ISSN 0377-9335

ENTOMON

Vol. 32 | June 2007 | No. 2

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Centre for Arthropod Bioresources and Biotechnology (CABB),
University of Kerala. Kariavattom, Trivandrum, India 695581

ENTOMON

ENTOMON is a quarterly journal of the Association for Advancement of Entomology issued in March, June, September and December, devoted to publication of research work on various aspects of insects and other arthropods.

Address MS and all editorial correspondence to Managing Editor, ENTOMON, Centre for Arthropod Bioresources and Biotechnology (CABB), University of Kerala, Kariavattom, Trivandrum 695581, India.

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ENTOMON 32(2): 71–78 (2007)
Article No. ent.32201

A simple method to isolate DNA from single specimen of onion thrips, *Thrips tabaci* Lindeman and melon thrips, *T. palmi* Karny (Thysanoptera: Thripidae) and molecular identification

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ABSTRACT: The tospoviruses belonging to Bunyaviridae are transmitted only by thrips vectors like *T. tabaci*, *T. palmi*, etc, which often cause 80% yield loss in vegetable crops like tomato, watermelon and onion. Identification of these vectors at early developmental stage is crucial since nymphs alone acquire the virus and the adults only transmit the disease. Morphological identification of these thrips at nymphal stage is often inconclusive, whereas molecular identification becomes handy and reliable. For successful molecular identification a simple quick method of DNA template preparation from single specimen of thrips is a prerequisite. Hence a simple, quick method of DNA template preparation from adult and nymph of *T. tabaci* and *T. palmi* has been developed. The DNA obtained in this method was used as template for molecular identification of the above species using primers specific to mitochondrial cytochrome oxidase I (mtCOI). The molecular identification had corroborated the morphological identification. The result of this investigation is useful in identification of thrips species especially at nymphal stage, a critical factor in understanding the epidemiology of the tospoviruses and their management.

KEYWORDS: *Thrips tabaci*, *T. palmi*, DNA isolation, molecular identification

INTRODUCTION

Thrips are important pests of wide range of agricultural and horticultural crops. They are serious in tropics on a number of crops, especially during summer. In the last two decades the emergence of exclusively transmitted new tospoviuses is responsible for loss amounting to billions of dollars worldwide (Ullman *et al.*, 2002). Till a couple

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of years back the Western flower thrips, *Frankliniella occidentalis* (Pergande) was considered as predominant vector of tomato spotted wilt virus (TSWV), a disease of pantomimic proportion. Recently the emergence of *T. palmi* as another major vector species of tospoviruses, especially in South and South East Asia is threatening vegetable cultivation. Further as vectors of tospoviruses such as watermelon bud necrosis virus (WBNV) transmitted by *T. palmi* and Iris yellow spot virus (IYSV) transmitted by *T. tabaci* they assume serious proportion in India (Ravi *et al.*, 2006). Identification of the insect vectors in the early developmental stages such as egg and nymph is very important in the vector-borne virus disease management. The above is very appropriate in the case of thrips vectors as nymphs can acquire the virus while the adults can only transmit (Ullman *et al.*, 2002; Mound, 2005; Whitfield *et al.*, 2005). Thrips species identification using conventional systematics requires adults for precision as the nymphs of different thrips species exhibit high level of similarity (Brunner *et al.*, 2002). On the other hand molecular systematics is not developmental stage specific including the sex of the test species. But successful species identification is limited by the availability of sufficient template from single specimen for polymerase chain reaction (PCR) for further cloning and sequencing. Even though methods are available for the isolation of DNA from single (Moritz *et al.*, 2000, 2001) and pool of specimens (Bayer *et al.*, 2001; Gyulai *et al.*, 2001; Brunner *et al.*, 2002) of different thrips species they involve many steps which require considerable time and to some extent expensive molecular biology chemicals. Therefore we have tested two single and quick single step procedures for the preparation of DNA template from adult and nymph of both *T. tabaci* and *T. palmi*. In this communication we report the usefulness of a particular method of DNA template preparation for molecular identification and corroboration with morphological identification.

MATERIALS AND METHODS

T. tabaci and *T. palmi* were collected from onion (Ark Niketan) and watermelon (Arka Manik), respectively, in the Indian Institute of Horticultural Research (IIHR), Bangalore. Pure cultures of both species were maintained on French bean pods (*Phaseolus vulgaris* cv Arka Komal) in plastic containers (10 cm × 10 cm) under room temperature. Adults and nymphs of *T. tabaci* and *T. palmi* from the pure culture were used for the molecular identification. Adults were identified by conventional systematics by Dr. Vikas Kumar, University of Delhi, South Campus, according to Bhatti (1980).

Two different methods of DNA template preparation were carried out from single specimen of adult and nymph of *T. tabaci* and *T. palmi*.

(A) Individual specimens in 0.5 ml PCR tubes containing 10 μl DNAase & RNAase free-water and ground thoroughly using sterile plastic micro pestle

(B) Individual specimens in 0.5 ml PCR tubes containing 10 μl DNAase & RNAase free water and sonicated (dr hiesches GmbH UP 100H, Germany) at 80% amplitude, 0.3 second cycle for 10 times.

For the above methods the tubes containing the homogenate were incubated in the boiling water for 5 minutes and immediately stored at −20 °C for 5 minutes and centrifuged at 8000 g for 5 minutes at 4 °C. Five μl of the supernatant was made use as template for PCR.

PCR was carried out in a thermal cycler (Primus 96, MWG Biotech, Germany) with the following cycling conditions; 94 °C for 3 minutes as initial denaturation followed by 40 cycles of 94 °C for 30 seconds, 53 °C for 45 seconds, 72 °C for 1 minute and 72 °C for 20 minutes as final extension. Since the mitochondrial cytochrome oxidase I (mtCOI) of animals exhibit more interspecies variation as compared to the other targets with primers mtD7.2F – 5'ATT AGG AGC HCC HGA YAT AGC ATT-3' & mtD9.2R – 5'GAG GCA AGA TTA AAA TAT AAA CTT CTG-3' resulting in amplification of around 500 bp PCR product (Brunner *et al.*, 2002) were used in the present study. PCR was performed in a 25 μl total reaction volume containing 20 Pico moles of each primer, 10 mM Tris-HCl (pH8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5U of Taq polymerase (Fermentas Life sciences). The amplified products were resolved in 1.5% agarose gels stained with ethidium bromide (10 μg/ml).

The PCR amplified fragments were eluted using Perfect prep® gel clean up according to the manufacturer's protocol (Eppendorf). The eluted PCR fragments were further ligated into the general purpose-cloning vector, InsT/Aclone (Fermentas Life sciences) according to the manufacturer's protocol. 5 μl of the ligated vectors were cloned into 200 μl of competent *Escherichia coli* (DH5α) cells. The above mixture was heat shocked at 42 °C for 45 seconds and the whole content was transferred into a tube containing 800 μl of SOC (tryptone—2% w/v, yeast extract—0.5% w/v, NaCl—8.6 mM, KCl—2.5 mM, $MgSO_4$—2.0 mM, Glucose—20 mM in 1000 ml water, pH7.0) and rotated at 150 rpm at 37 °C for 1 hour. 200 μl of the above culture was spread on Luria Bertani agar (LBA) (tryptone—10 g, yeast extract—5 g, NaCl—5 g, agar—15 g in 1000 ml of water, pH 7.0) containing ampicillin (100 g/ml), IPTG (4 μg/ml) and X-gal (40 μg/ml) and were incubated at 37 °C for 16 hours. Blue/White selection was carried out and all the white colonies were maintained on LBA containing ampicillin (100 μg/ml), incubated at 37 °C overnight and stored at 4 °C until further use.

Plasmids were prepared from the overnight culture of the white colonies cultured in LB broth (enzymatic casein—10 g, yeast extract—5 g, NaCl—5 g in 1000 water, pH 7.0) using modified alkali lysis method (Brinboim and Doly, 1979) and were resolved in 1.0% agarose gel, stained with ethidium bromide (10 μg/ml). Clones that showed appropriate molecular weight (2.3 kb) as compared to control plastid (1.8 kb) were used for sequencing. For the purpose of sequencing plasmids were prepared using plasmid kit mini (Qiagen) from five selected clones for each adult and nymph for both *T. tabaci* and *T. palmi* in order to find out intra individual variations and sequencing errors, if any. Sequencing was carried out in an automated sequencer (ABI Prism 310) using M13 universal primers both in forward and reverse directions. The sequences

FIGURE 1. Standardization of DNA isolation from adult and nymph of *Thrips tabaci* and *T. palmi*. **Method A** – Grinding; **Method B** – Sonication.

M – Molecular weight marker (1 kb ladder)

Lane 1 – PCR amplified product from *T. tabaci* adult (method A)
Lane 2 – PCR amplified product from *T. tabaci* nymph (method A)
Lane 3 – No amplification in sonicated *T. tabaci* adult (method B)
Lane 4 – No amplification in sonicated *T. tabaci* nymph (method B)
Lane 5 – PCR amplified product from *T. palmi* adult (method A)
Lane 6 – PCR amplified product from *T. palmi* nymph (method A)
Lane 7 – No amplification in sonicated *T. palmi* adult (method B)
Lane 8 – No amplification in sonicated *T. palmi* nymph (method B)

were aligned in the bioinformatics software, Bioedit and homology search was done using BLAST (`http://www.ncbi.nlm.nih.gov`).

RESULTS AND DISCUSSION

A single fragment of about 500 bp was amplified from adult and nymphal DNA templates in the above methods (A & B) for both *T. tabaci* and *T. palmi* (Fig. 1). There was no amplification in sonication method for both stages in the above thrips species. Close observation of the sonicated homogenate showed that the specimens were not ground properly which would have inhibited the release of DNA during boiling resulting in no amplification. Sonication method has not been tried for isolation of DNA template from thrips species till date. Researchers employed various protocols for the isolation of DNA from thrips species viz. regular method of DNA isolation viz. homogenization, extraction, precipitation, resuspension, etc (Brunner *et al.*, 2002; Toda and Komazaki, 2002; Frey and Frey, 2004). Normally the above methods of DNA isolation required more than 1 hour. The present method required just about 20 minutes for DNA template preparation both from adult and nymphal stages in both test species. In addition, this method does not require any other molecular biology chemicals and reagents input. Usually various parameters like extent of sclerotization,

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                              10        20        30        40        50        60        70        80        90       100
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips tabaci-adult  ATTAGGAGCACCTGACATAGCATTCCCTCGATTAAATAATATAAGATTCTGACTTTTACCCCCTTCTCTGGGATTATTAATTATAGGACTTTATAAAGAA
Thrips tabaci-nymph  ATTAGGAGCACCTGACATAGCATTCCCTCGATTAAATAATATAAGATTCTGACTTTTACCCCCTTCTCTGGGATTATTAATTATAGGACTTTATAAAGAA

                             110       120       130       140       150       160       170       180       190       200
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips tabaci-adult  GGAGCGGGAACGGGATGAACAGTATATCCACCTTTATCAACGTTTTATCATTCAGGACCTTCAGTAGACTTAACAATTTTTTCTTTACACCTTGCAGGGA
Thrips tabaci-nymph  GGAGCGGGAACGGGATGAACAGTATATCCACCTTTATCAACGTTTTATCATTCAGGACCTTCAGTAGACTTAACAATTTTTTCTTTACACCTTGCAGGGA

                             210       220       230       240       250       260       270       280       290       300
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips tabaci-adult  TTTCTTCAATTTTAGGTGCCTTAAATTTTATTACTACAATTATTAATCTTAAAGCAAAAAACCTTTCAGCAGAAAAAATTAGACTATTTGTCTGATCAGT
Thrips tabaci-nymph  TTTCTTCAATTTTAGGTGCCTTAAATTTTATTACTACAATTATTAATCTTAAAGCAAAAAACCTTTCAGCAGAAAAAATTAGACTATTTGTCTGATCAGT

                             310       320       330       340       350       360       370       380       390       400
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips tabaci-adult  TATTTTAACAGCCATTCTTCTTCTTTTATCTTTGCCAGTGTTAGCGGGAGCTATCACAATACTTTTAACTGACCGAAACTTAAATACCTCTTTTTTTGAC
Thrips tabaci-nymph  TATTTTAACAGCCATTCTTCTTCTTTTATCTTTGCCAGTGTTAGCGGGAGCTATCACAATACTTTTAACTGACCGAAACTTAAATACCTCTTTTTTTGAC

                             410       420       430       440       450       460       470       480
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips tabaci-adult  CCTAGAGGGGGAGGGGACCCTGTTTTATATCAACACCTTTTTTGATTTTTTGGTCACCCAGAAGTTTATATTTTAATCTTGCCTG
Thrips tabaci-nymph  CCTAGAGGGGGAGGGGACCCTGTTTTATATCAACACCTTTTTTGATTTTTTGGTCACCCAGAAGTTTATATTTTAATCTTGCCTG
```

FIGURE 2. Partial mitochondrial cytochrome oxidase I (mtCOI) gene sequence for adult and nymph of *T. tabaci*.

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                               10        20        30        40        50        60        70        80        90       100
                       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips palmi - adult   ATTAGGAGCACCTGACATAGCATTCCCACGATTAAATAACATAAGATTTTGACTTCTTCCACCCTCTTTAACTCTTTTAATTATAGGTTTATATAAAGAA
Thrips palmi - nymph   ATTAGGAGCACCTGACATAGCATTCCCACGATTAAATAACATAAGATTTTGACTTCTTCCACCCTCTTTAACTCTTTTAATTATAGGTTTATATAAAGAA

                              110       120       130       140       150       160       170       180       190       200
                       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips palmi - adult   GGAGCGGGAACAGGATGAACAGTCTGTCCACCTTTATCAACATTTTACCATGCTGGTATTTCAGTAGACTTAACAATCTTTTCTCTTCATTTAGCTGGAG
Thrips palmi - nymph   GGAGCGGGAACAGGATGAACAGTCTGTCCACCTTTATCAACATTTTACCATGCTGGTATTTCAGTAGACTTAACAATCTTTTCTCTTCATTTAGCTGGAG

                              210       220       230       240       250       260       270       280       290       300
                       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips palmi - adult   TCTCCTCAATTTTAGGAGCATTAAATTTCATTACTACAATTTTAAATTTAAAAAATAAAAATTTATCAAGAGAAAAATTAAGATTATTTGTGTGGTCAGT
Thrips palmi - nymph   TCTCCTCAATTTTAGGAGCATTAAATTTCATTACTACAATTTTAAATTTAAAAAATAAAAATTTATCAAGAGAAAAATTAAGATTATTTGTGTGGTCAGT

                              310       320       330       340       350       360       370       380       390       400
                       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips palmi - adult   AATATTAACAGCAATTCTTTTACTTTTATCTTTACCTGTACTTGCAGGAGCCATTACAATACTTTTAACAGACCGTAATTTAAACACGTCATTCTTTGAC
Thrips palmi - nymph   AATATTAACAGCAATTCTTTTACTTTTATCTTTACCTGTACTTGCAGGAGCCATTACAATACTTTTAACAGACCGTAATTTAAACACGTCATTCTTTGAC

                              410       420       430       440       450       460       470       480
                       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Thrips palmi - adult   CCAAGAGGGGGAGGAGATCCTGTACTTTATCAACATCTATTCTGATTCTTTGGGCATCCAGAAGTTTATATTTTAATCTTGCCTG
Thrips palmi - nymph   CCAAGAGGGGGAGGAGATCCTGTACTTTATCAACATCTATTCTGATTCTTTGGGCATCCAGAAGTTTATATTTTAATCTTGCCTG
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FIGURE 3. Partial mitochondrial cytochrome oxidase I (mtCOI) gene sequence for adult and nymph of *T. palmi*.

size, etc affects efficient DNA isolation. Since the present method has been found to be suitable for thrips that are minute and having relatively tough exoskeleton the same method can be tested on other insects. Moreover this method could be followed in labs which are less equipped. Availability of quick and easy method of DNA isolation is a prerequisite for high throughput for which the present method is a suitable one.

Multiple alignment of the sequences from adult and nymphal stages for each test species of thrips showed that there was no intra specific variation both in *T. tabaci* and *T. palmi* (Figs. 2 and 3). But Bayer *et al.* (2002) and Frey and Frey (2004) observed intra species variations in *T. tabaci*. Therefore we conclude that the specimens selected for our study in each species were homogenous. Further, BLAST search showed that sequences from adult and nymphal stages for each thrips species matched the respective species without any ambiguity. Since there is no change in the sequences obtained from nymphal stages as compared to the adults, nymphs could be successfully used for species identification. Therefore the present method is quick; simple yet can be successful in isolating DNA from single specimen of both adult and nymphal stages. In addition to the above the results facilitate studying the host and location associated genetic differences (Brunner *et al.*, 2004) in different thrips populations.

ACKNOWLEDGEMENT

The authors are grateful to the Director, Indian Institute of Horticultural Research, Bangalore 560089 for encouragement and facilities.

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(Received 20 July 2006; accepted 15 May 2007)

ENTOMON 32(2): 79–88 (2007)
Article No. ent.32202

A taxonomic study of *Sphegigaster* Spinola (Hymenoptera: Pteromalidae) from Yemen

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ABSTRACT: Three new species of *Sphegigaster* viz. *S. scutaecus*, *S. diasi* and *S. trioni* are described from Yemen. The genus *Sphegigaster* is reported for the first time from Yemen. A key to Yemen species of *Sphegigaster* is also provided.

KEYWORDS: Hymenoptera, Pteromalidae, new species, Yemen

INTRODUCTION

Though the genus *Sphegigaster* Spinola contains several species reported from all the continents, no species is recorded so far from Yemen. One of us (A. v. H.) has made extensive collection of several genera of Chalcidoidea from Yemen. While studying these specimens we came across three new species and a known species of *Sphegigaster* from Yemen. The new species do not fit to the description of any known old world species listed by Noyes (2005). The new species are described below and the known species is commented upon. A key to species of *Sphegigaster* of Yemen is also provided. All the types are deposited at DZUC pending transfer to ZSIC very soon.

ABBREVIATIONS USED

CC = Costal cell; F1–F7 = Funicular segments 1 to 7; MS = Malar sulcus; MV = Marginal vein; OOL = Ocellocular distance; PMV = Postmarginal vein; POL = Postocellar distance; SMV = Submarginal vein; STV = Stigmal vein; T1-T2 = Gastral tergites 1-2; DZUC = Department of Zoology, University of Calicut; ZSIC = Zoological Survey of India, Kolkatta (= Calcutta).

KEY TO THE SPECIES OF *SPHEGIGASTER* OF YEMEN (BASED ON FEMALES)

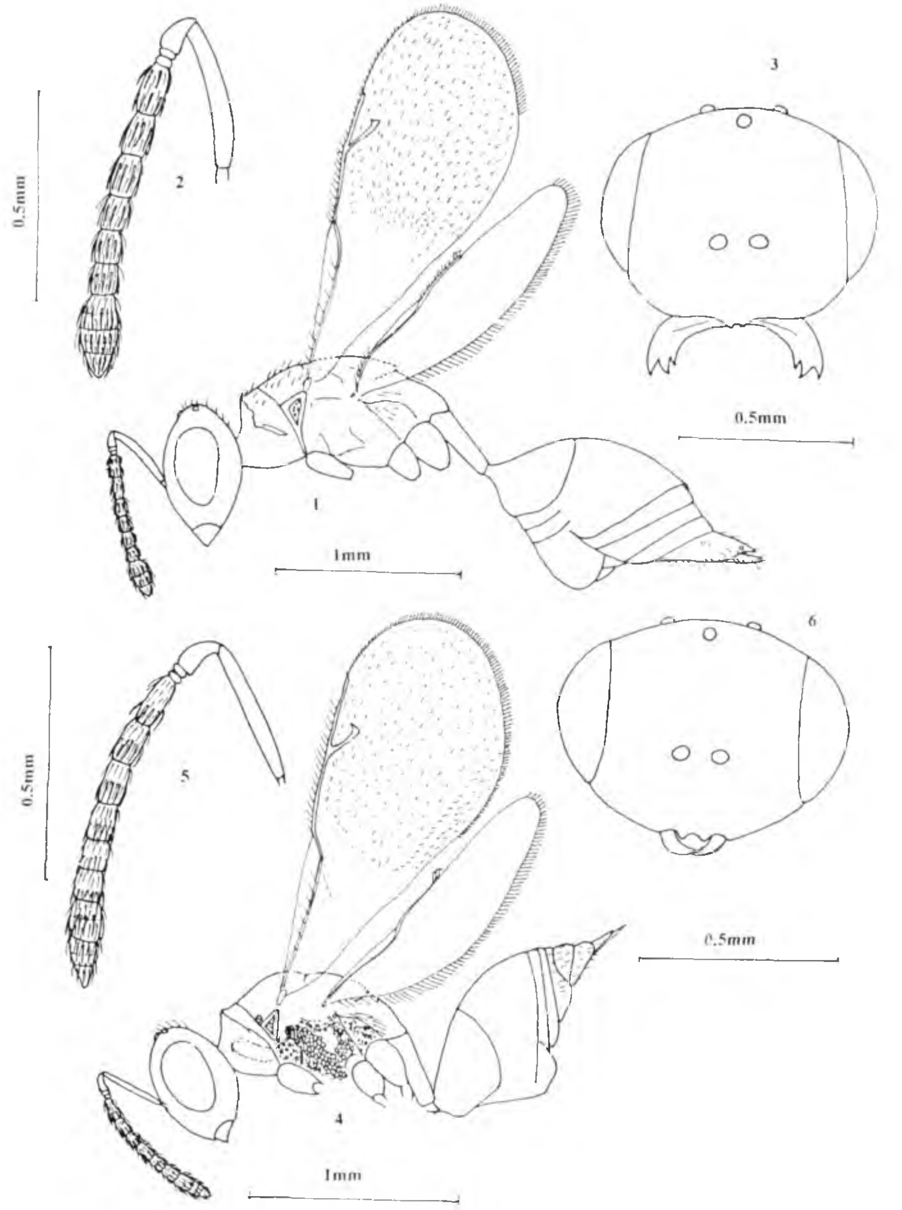
1. Antennal scape black with or without metallic green refringence 2
= Antennal scape yellowish brown without metallic green refringence 3
2. Antennal scape without metallic green refringence; hind margin of T1 with its middle portion slightly curved; MV 3x as long as STV, 1.36x as long as PMV; petiole 1.43x as long as propodeum; propodeal spiracle separated from metanotum by nearly its own diameter; hypopygium just reaching middle of gaster .... *DIASI* Narendran & van Harten sp. nov.
= Antennal scape with metallic green refringence; hind margin of T1 with its middle portion not curved; MV 1.8x as long as STV; 1.5x as long as PMV; petiole 1.64x as long as propodeum; propodeal spiracle separated from metanotum by less than half its diameter; hypopygium exceeding a little over middle of gaster .... *SCUTAECUS* Narendran & van Harten sp. nov.
3. MV 2.5x as long as STV; PMV 1.9x as long as STV; hind margin of T1 slightly curved posteriorly; scape 2.25x as long as F1 ; petiole of gaster nearly 2x as long as broad .... *CUSCUTAE* Ferriere
= MV 1.88x as long as STV; PMV 1.44x as long as STV; hind margin of T1 not curved posteriorly; scape 3.3x as long as F1; petiole of gaster 2.3x as long as broad............................ *TRIONI* Narendran & van Harten sp. nov.

1. *Sphegigaster scutaecus* Narendran & van Harten sp. nov. (Figs. 1–3)

Holotype Female

Length 3.18 mm. Black with slight metallic green reflections, except the following parts: eye brown; ocelli pale reflecting yellow; antenna black with metallic green refringence on scape; mandibles pale yellowish brown with base and teeth brown; tegula pale yellow, legs with coxae concolorous with mesosoma; trochanters brown; femora brown with apices paler; tibiae pale yellow; tarsi with first three segments pale yellow, gradually becoming dark brown; ventral side of hypopygium pale brown; forewing hyaline, veins pale yellowish brown; pilosity pale brown.

Head: 1.27x as wide as long (excluding mandibles) in anterior view, 2.78x as wide as its median length in dorsal view; POL 1.2x OOL; eye height in side view 1.8x its width, 4.5x as long as malar space, eyes separated by 1.52x their own length; malar space 0.22x eye height in profile; mandibles quadridentate. Antenna inserted well above the level of ventral edge of eyes; scape reaching anterior ocellus, its length a little shorter than eye height in profile, greater than transverse diameter of eye; combined length of pedicellus plus flagellum subequal to head width in dorsal view; pedicellus 2x as long as broad, 0.63x as long as anelli plus F1; pedicellus stouter than F1; funicle thicker towards distad, with segments 1 to 6 longer than wide; clava 2x as long as wide, 0.57x length of scape, distinctly longer than preceding two segments combined (20: 15); sensillae as in Fig. 2.



FIGURES 1–3. *Sphegigaster scutaecus* Narendran & van Harten sp. nov. (Female)

1. Body profile (in part); 2. Antenna; 3. Head, anterior view.

FIGURES 4–6. *Sphegigaster diasi* Narendran & van Harten sp. nov. (Female)

4. Body profile (in part); 5. Antenna; 6. Head anterior view.

Mesosoma: Pronotum with lateral angles slightly toothed, its anterior edge slightly ridged; somewhat wavy; mesoscutum 2.22x as broad as long, with raised reticulations; scutellum a trifle shorter than wide (9:10), 0.75x as long as mesoscutum, sculptured as on mesoscutum; frenal line hardly distinct in the middle; frenum with reticulations as on rest of scutellum. Propodeum 0.56x length of scutellum, sculptured as on scutellum, median carina absent; spiracles small, oval, separated from metanotum by less than half its diameter; callus densely pilose; meso and metapleura densely reticulate. Forewing 2.45x as long as broad; CC with a single row of hairs which becomes double in the distal half; basal; cell bare; both it and speculum open behind; speculum extends to apical side below MV; disc of forewing beyond speculum moderately pilose; MV 1.8x STV; 1.5x PMV.

Gaster: Petiole 1.64x as long as propodeum and exceeding well beyond tips of hind coxae, 2.5x as long as broad, with strong raised reticulations, becoming narrower towards posterior end. Gaster longer and narrower than mesosoma, 3.1x as long as broad in dorsal view; hind margin of T1 truncate medially; T2 longest, 1.21x as long as broad, 2.12x as long as T1; hypopygium exceeding a little over middle of gaster.

Male

Differs from female in having: Body with brighter metallic green refringence; scape not reaching front ocellus; flagellum sub cylindrical, segments differ slightly from those of female; gaster relatively much shorter.

Host

Unknown.

Holotype

Female, YEMEN, Sana'a. ii. 1991, A. van Harten (DZUC).

Paratypes

1 Female, YEMEN, Sana'a. ii.1991, A. van Harten; 1 Female, YEMEN, Sana'a. vii.1991, A. van Harten; 1 Female, YEMEN, Sana'a., ii. 1992, A. van Harten; 1 Female, YEMEN, Sana'a. v. 1999, A. van Harten.

Etymology

Arbitrary combination of letters.

DISCUSSION

This new species comes near *S. glabrata* Graham in the key to species by Graham (1969), but *S. glabrata* differs from this new species in having: 1) Head and mesosoma

dark bluish green (in *S. scutaecus* head and mesosoma black with metallic green refringence); 2) antennal scape brown (in *S. scutaecus* scape black with metallic green refringence); 3) eye 1.5x as long as broad (in *S. scutaecus* eye 1.8x as long as broad); 4) eyes separated by about 1.35x their own length (in *S. scutaecus* eye separated by about 1.52x their own length); 5) malar space 0.33x length of eye (in *S. scutaecus* malar space 0.22x length of eye); 6) pedicellus nearly equal to the anelli plus F1 (in *S. scutaecus* pedicellus 0.63x anelli plus F1); 7) clava 2.5x as long as preceding 2 segments (in *S. scutaecus* clava 1.33x preceding 2 segments), and in several other features. The new species does not fit to key of Sureshan (2003).

2. *Sphegigaster diasi* Narendran & van Harten sp. nov. (Figs. 4–6)

Holotype Female

Length 2.19 mm. Black with slight metallic green refringence, except the following parts: eye pale yellowish brown; ocelli pale reflecting yellow; antenna dark brown except slightly paler pedicel, scape black without metallic green reflection; tegula pale brownish yellow; legs with coxae concolorous with mesosoma; remaining segments pale brownish yellow with fourth tarsal segments and pretarsi dark brown. Wings hyaline with veins pale brownish yellow; pilosity of wings pale white.

Head: 1.28x as wide as long (excluding mandibles) in anterior view, 2.92x as wide as its median length in dorsal view; temples converging 0.8x as long as eye length in dorsal view; POL 1.14x OOL; eye height in side view 1.54x its width, 4.25x as long as malar space, eyes separated by 1.29x their own length; malar space 0.24x eye height in profile. Antenna inserted well above the level of ventral edge of eyes; scape reaching anterior ocellus, its length a little shorter than eye height in profile, greater than transverse diameter of eye; combined length of pedicellus and flagellum as long as width of head in dorsal view; pedicellus 2x as long as broad, 0.63x as long as anelli plus F1; funicle proximally not stouter than the pedicellus but thickening distad, with segments 1 to 5 slightly elongate; sixth quadrate; clava 2.5x as long as broad, 0.59x length of scape, distinctly larger than preceding two funicular segments (20: 17); sensilla distributed as in Fig. 5.

Mesosoma: Pronotum with a distinct carinate ridge, lateral angle not distinctly toothed; mesoscutum 2.4x as broad as long, with raised reticulations; scutellum 1.17x as broad as long, slightly convex; sculptured as the mesoscutum; the frenal line slightly indicated in the middle, strongly indicated in sides, frenum reticulate. Propodeum 0.56x length of scutellum, sculptured as on scutellum, median carina absent, spiracles rather small, oval, each spiracle separated by nearly its own length from metanotum; callus alutaceous, densely pilose; metapleuron strongly reticulate, mesopleuron finely and strongly reticulate. Forewing 2.34x as long as broad, relatively sparsely pilose; lower surface of CC with a single row of hairs, partly double in the distal quarter, its upper surface bare; basal cell bare, both it and speculum open below; on the upper surface of the wing the speculum large and extend as a bare strip below MV; disc of

wing beyond speculum relatively sparsely pilose; MV 3x STV, 1.36x as long as PMV; legs not very slender; spur of midtibia 0.4x length of midmetatarsus.

Gaster: Petiole 1.43x as long as propodeum and exceeding well beyond tips of hind coxae, 3x as long as broad. strongly reticulate, its sides converging very slightly posteriorly. Gaster ovate, longer and narrower than mesosoma, 2.86x as long as broad; T1 occupying one-third the total length; hind margin of T1 curved in the middle; T2 longest, 1.27x as long as broad; hypopygium just reaching middle of gaster.

Male

Differs from female as follows: Body with more bright metallic green refringence; scape not quite reaching front ocellus, its length distinctly less than the transverse diameter of eye; combined length of pedicellus plus flagellum 1.36x breadth of head; flagellum cylindrical; sensilla numerous; gaster shorter than mesosoma.

Host

Unknown.

Holotype

Female, YEMEN, N. Sana'a., 5.vi.1998, A. van Harten (DZUC).

Paratypes

2 Females, YEMEN, N. Sana'a., ix.1992, A. van Harten; 1 Female, YEMEN, N. Sana'a., iii.1991. A. van Harten; I Female, vii.1991, YEMEN, N. Sana'a. 5.vi.1998, A. van Harten (DZUC).

Etymology

Arbitrary combination of letters.

DISCUSSION

This new species comes near *Sphegigaster brevicornis* (Walker) in the key to species by Graham (1969) but *S. brevicornis* differs from this new species in having: 1) Forewing more densely pilose than this new species; 2) Petiole 1.5x as long as broad (in *S. diasi* petiole 3x as long as broad); 3) Mesoscutum nearly or quite 2x as long as broad (in *S. diasi* mesoscutum distinctly more than 2x as long as broad); 4) Gaster maximum up to 2.5x as long as broad (in *S. diasi* gaster 2.86x as long as broad); 5) Pronotal collar almost rounded anteriorly (distinctly margined anteriorly in *S. diasi*).

This new species comes near *S. anamudiensis* Sureshan and Narendran in the key to species of *Spegigaster* by Sureshan and Narendran (1997) and Sureshan (2003), but *S. anamudiensis* differs in having: 1) F1 not narrowed basally (slightly narrowed

basally in *S. diasi*); 2) Female body bluish green with slight golden reflection (in *S. diasi* body black with slight metallic green reflection in female); 3) Head 2x as wide as its length in anterior view (2.92x as wide as long in *S. diasi*); 4) Malar space 0.47x length of eye (in *S. diasi* malar space 0.24x as long as eye); 5) Basal cell of forewing with setae (not so in *S. diasi*); 6) MV 2.8x STV (in *S. diasi* MV 3.75x STV).

3. *Sphegigaster trioni* Narendran & van Harten sp. nov. (Figs. 7–9)

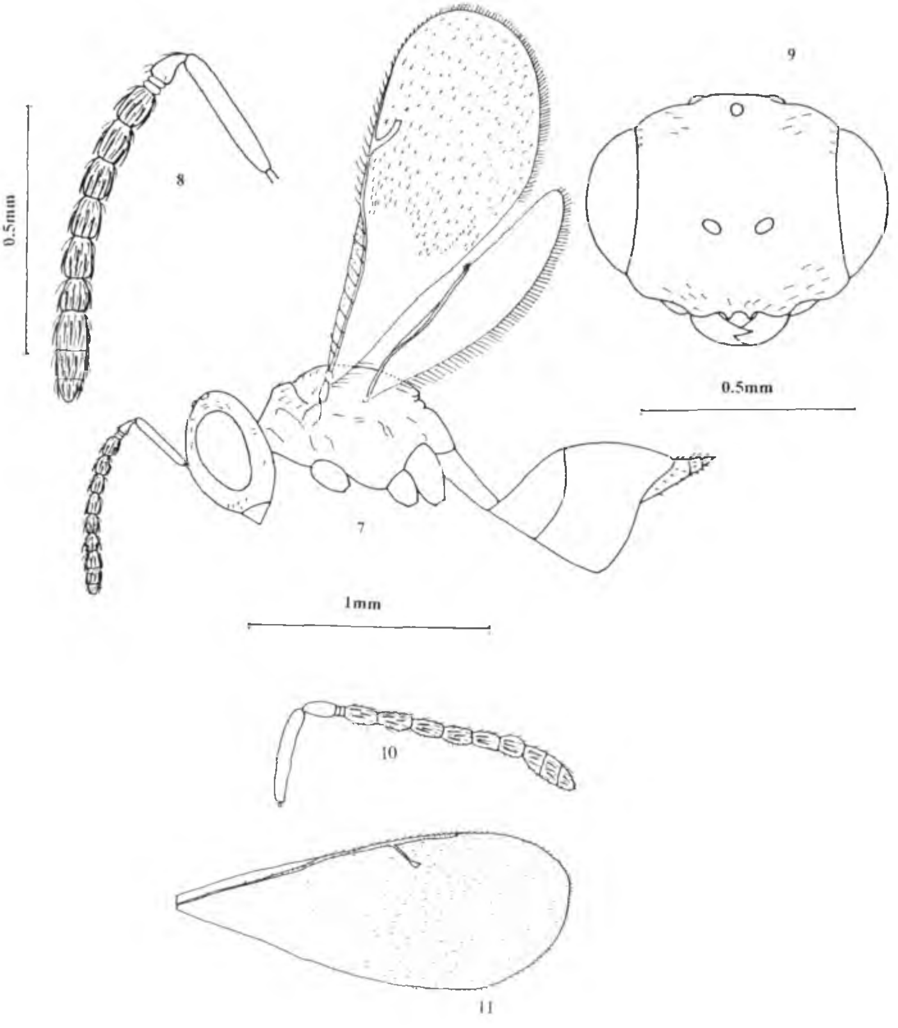
Holotype Female

Length 2.14 mm. Black with slight metallic bluish green refringence, except the following parts: eye reddish brown with paler margin around; ocelli pale reflecting yellow; antennae dark brown with pedicel and scape pale yellowish brown; tegula pale yellow; all coxae concolorous with mesosoma, remaining segments of legs pale yellow; wings hyaline, veins pale brownish hyaline.

Head: Width in anterior view 1.31x as broad as its length (excluding mandibles), 3.1x as broad as its median length in dorsal view; temples converging 0.7x as long as eye in dorsal view; POL as long as OOL; eye height in side view 1.67x its width, 5x as long as malar space; eyes separated from each other 1.38x their own length in anterior view; malar space 0.2x eye height in profile. Antenna inserted well above lower level of eyes; scape slightly exceeding level of vertex, shorter than eye height (17: 20); combined length of pedicellus plus flagellum 1.1x head width in dorsal view; pedicellus 1.5x as long as broad, 0.69x as long as anelli plus F1; funicular segments as in figure 8; clava 3.14x as long as broad, 0.67x length of scape, 1.22x combined length of preceding two segments.

Mesosoma: Pronotum with a carinate ridge, lateral angle not distinctly toothed; mesoscutum 2.44x as broad as its length; scutellum as long as mesoscutum, a little wider than long (10: 9); pronotum, mesoscutum, scutellum and propodeum distinctly and densely reticulate; propodeum 0.78x as long as length of scutellum, median carina absent; propodeal spiracle oval, separated from metanotum by its own diameter; callus alutaceous, densely pilose; metapleuron reticulate, mesopleuron finely and strongly reticulate. Forewing 2.3x as long as broad, moderately pilose; lower surface of CC with a single irregular row of hairs, basal cell bare; speculum open below, not quite extending to STV below MV; MV 1.88x as long as STV, a little shorter than PMV (16.5: 18); basal vein bare, spur of midtibia 0.42x as long as midmetatarsus.

Gaster: Petiole 1.42x as long as propodeum and exceeding well beyond tip of hind coxa, 2.3x as long as broad in dorsal view, slightly narrowing posteriorly, with two setae on either side proximally. Gaster ovate, longer and narrower than mesosoma, 2x as long as broad, T1 and T2 as in figure 7; T2 covering most of the remaining tergites; hypopygium exceeding middle of gaster.



FIGURES 7–9. *Sphegigaster trioni* Narendran & van Harten sp. nov. (Female).
7. Body profile (in part); 8. Antenna; 9. Head anterior view.
FIGURES 10–11. *Sphegigaster cusctuae* Ferriere (Female)
10. Antenna; 11. Forewing.

Male

Unknown

Host

Unknown.

Holotype

Female, YEMEN, N. Sana'a., 2.iii.1998, A. van Harten (DZUC).

Etymology

Arbitrary combination of letters.

DISCUSSION

This new species comes near *S. glabrata* Graham in the key to species by Graham (1969), but *S. glabrata* differs from this new species in having: 1) Forewing with speculum extending to STV below MV as a narrow strip; 2) Pedicel longer than F1; 3) In different proportion of length between antennal segments, besides several other characters.

4. *SPHEGIGASTER CUSCUTAE* FERRIERE (FIGS. 10–11)

Sphegigaster cuscutae Ferriere, 1959: 98–99.

Diagnosis

Female

Length 1.9–2.3 mm. Black with metallic green refringence; antennal scape pale yellowish brown; legs pale yellowish brown except coxae. Coxae brownish black with slight metallic green refringence. Antenna (Fig. 10) with flagellum slightly widening from F1 to clava; clava much less than 2x as broad as F1; funicular segments longer than broad except F6 which is subquadrate; scape reaching front ocellus; pedicel subequal or equal in length to F1. Pronotum with weak anterior margin and feeble denticle on either side. Forewing disc sparsely pilose (Fig. 11), basal cell bare; relative lengths of veins: SMV = 45; MV = 22; PMV = 17; STV = 9. Metasoma with petiole nearly 2x as long as broad; hind margin of T1 slightly curved posteriorly (in some specimens hardly curved); ovipositor sheath a little exserted.

Male

Length 1.6–2 mm. Pronotal collar with distinct teeth; hairs on flagellum as long as width of segments that bear them (as in figure of Ferriere, 1959: 98).

Host

Puparia of *Melanagromyza cuscutae* Her. (Graham, 1969).

Material examined

15 Females and 8 Males, YEMEN: Sana'a, 1 Female, Tali zz. All specimens collected by A. v. Harten (DZUC).

ACKNOWLEDGEMENTS

The first author (T. C. N.) is grateful to the University of Calicut for facilities to work. He also thanks his students Miss. M. Sheeba and Miss. M. C. Jilcy for assisting in the preparation of this paper. The junior author thanks his Yemeni colleagues Mohamed Mahyoub and Ahmed Seif Al Absi for their assistance with the operation of the traps.

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(Received 30 October 2006; accepted 15 May 2007)

ENTOMON **32(2)**: 89–95 (2007)
Article No. ent.32203

Host plant-based morphological, ecological and esterase variations in *Aphis gossypii* Glover populations (Homoptera: Aphididae)

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ABSTRACT: The aphid species, *Aphis gossypii* Glover shows wide variations in its fecundity and population dynamics on different host plants in various parts of India. Clones of *A. gossypii* were raised in laboratory, in Tripura on four different host plants. The insect clones showed variations in their morphology, growth parameters and esterase isozymes. The clones on cotton were found to be largest and showed higher growth rates than the clones on other host plants.

KEYWORDS: Host plant specialisation, aphids, *Aphis gossypii*

INTRODUCTION

Species which reproduce by asexual means are often distributed widely and are ecologically more diverse (Lynch, 1984). High variability, both genetical and ecological, in the populations of such species is caused by their adaptation to patchy habitats, host plants in case of aphids (Wohrmann and Hales, 1989). Among aphids, *Aphis gossypii* Glover is the perfect example of such species as its asexual populations show clonal diversities in relation to host-plants in several parts of the world (Inaizumi, 1981; Guldemond *et al.*, 1994; Wool and Hales, 1996; Fuller *et al.*, 1999). In India, *A. gossypii* affects a wide range of agricultural and horticultural plants and is considered a serious pest of crops belonging to Cucurbitaceae, Malvaceae and Solanaceae (Agarwala and Ghosh, 1985; Rai *et al.*, 1990; Panchabhavi *et al.*, 1990). Population diversity in asexual populations of aphids often creates problems in taxonomy, identification and management of pest populations (Miyazaki, 1987; Raychaudhuri, 1980). It was therefore felt desirable to understand the nature of variability in the Indian populations of this species.

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The aim of the present study was to determine the effect of some of the common host-plants in India on the morphology and growth parameters in *A. gossypii*. Variations in some of its metabolic enzymes in such clones also were studied.

MATERIALS AND METHODS

Live samples of *A. gossypii* were collected from farmlands in the neighbourhood of the Tripura University, 12 km south of Agartala city. Collections were made from cultivated varieties of cotton (*Gossypium hirsutum*), brinjal (*Solanum melongena*), arum (*Colocasia esculenta*) and chilli (*Capsicum annuum*). Parthenogenetic reproductive females collected from these plants were used to develop clones on the respective host plants under greenhouse conditions. Saplings of these host plants were maintained individually in 10 cm diameter pots (20 cm in case of cotton plants). Each plant was enclosed in Terylene gauze supported by a wooden frame to prevent entry of other aphid clones. All the clones were allowed to attain the carrying capacity of individual plants.

Ten adult specimens of apterous morph (1-day old) were collected from each of the *A. gossypii* clones in the greenhouse. Whole-mounts were prepared following the procedure described by Raychaudhuri (1980). Taxonomically important characters viz. length of body (BL), antenna (ANT), antennal segments III and VI (ANT III, VI), proboscis (PROB), siphunculus (SIPH), cauda (CAU) and ultimate rostral segments (URS) were measured under a microscope having ocular micrometer.

Population growth rate (GR), which is the change in the number of individuals in population per unit time, was recorded in the rising phase of population increase and was calculated using the following equation: $GR = (N_t - N_o)/\Delta t$, where N_o is the number of aphids initially released on a potted plant, N_t is the number of aphids recorded at the maximum count or carrying capacity of the plant, and Δt is the difference of time between N_o and N_t.

Mean relative growth rate (MRGR), which is a measure for assessing the performance of different clones of the same species, was calculated following the method of Watt and Hales (1996). $MRGR = \sum(\log_{10}$ adult weight $- \log_{10}$ birth weight)/developmental time, expressed as $\mu g \mu g^{-1}\ d^{-1}$ (μg increase in weight per μg of aphid per day).

Carrying capacity (K), which is the upper limit of population size of an organism that is acceptable to a given environmental condition, was determined using the equation: $K = \sum(N_{max} - N_{min})/N_{max}$, where K is the carrying capacity of the individual host plant, and N_{max} and N_{min} are the maximum and minimum number of aphids, respectively, present in the population at the beginning and at the peak of growth. Time taken to reach the carrying capacity, Tk, was also calculated by the equation: $Tk = \sum$ no. of days to K/n, where n is the number of observations.

For electrophoretic study homogenates of each sample were prepared from 15 mg of live aphids of respective clones from different host plants in a mixture of 0.025 M sucrose and 0.10 M TRIS-HCl extraction buffer (pH 6.8) in 1:1 ratio. Individual samples were centrifuged at 10000 rpm for 20 minutes at 6 °C. 25 μl of each supernatant was loaded into a 8% polyacrylamide slab gel pre-soaked in electrode

TABLE 1. Variation in morphological characters of apterous *A. gossypii* clones obtained from four host plant species

Characters	Mean of measurements in mm (n = 10)			
	Cotton	Brinjal	Chilli	Arum
BL	1.09^{a}	0.93^{b}	0.79^{c}	0.87^{bd}
ANT	0.74^{a}	0.65^{b}	0.56^{c}	0.61^{bcd}
ANT III	0.16^{a}	0.17^{a}	0.16^{a}	0.16^{a}
ANT VI	0.30^{a}	0.29^{a}	0.26^{b}	0.28^{ab}
SIPH	0.20^{a}	0.15^{b}	0.13^{c}	0.14^{bcd}
CAU	0.16^{a}	0.09^{b}	0.08^{c}	0.09^{bcd}
URS	0.08^{a}	0.07^{b}	0.06^{c}	0.07^{bd}
PROB	0.35^{a}	0.29^{b}	0.27^{c}	0.31^{ad}

Dissimilar alphabets with mean values in a row indicate significant differences by Tukey's multiple range test.

buffer (1.5 g TRIS-HCl, 17.3 g glycine, pH 8.3) using a 7-lane vertical electrophoretor. Electrophoresis was carried out at 16 mA constant current for about two hours in a refrigerator at 4 °C. Prior to staining, gels were kept in enzyme buffer solution for 40 minutes. Thereafter, gels were transferred to a reaction mixture at 37 °C for 30 minutes. Reaction mixture was prepared according to the procedure described by Loxdale *et al.* (1983) and Singh and Cunningham (1981). The gels were read on an illuminated table. Bands were marked in order of increasing anodal mobility (RM: relative mobility to bromophenol blue). Variations in the position of bands in the gel and their intensity of staining were recorded for each aphid clone. In order to record possible variation due to the effects of laboratory procedure of aphid rearing and electrophoresis, three separate gels were prepared from each aphid clone.

Differences in morphometry and growth parameters in different *A. gossypii* clones were analyzed with Tukey's multiple range test.

RESULTS

Morphological variations

Results presented in Table 1 show that aphid clones from chilli and arum plants were significantly smaller than those from brinjal and cotton plants in respect of length of body, antennae and siphunculi. Aphid clones from chilli plants were the smallest and those from cotton plants were the largest among the four host plant-based clones of this study. Antennal segment III and ultimate rostral segments showed least variations whereas length of body and total length of antennae showed maximum variations between the clones. Ultimate rostral segments, though, showed minimum variation between the smallest and largest aphids infesting chilli and cotton plants, respectively, but the length of proboscis showed considerable variation between them.

TABLE 2. Variation in growth parameters of apterous *A. gossypii* clones obtained from four plant species

Growth parameter	Mean of measurements (n = 10)			
	Cotton	Brinjal	Chilli	Arum
GR (increase in aphid no/day)	45.58^{a}	4.92^{b}	18.46^{c}	8.60^{bd}
MRGR ($\mu g\ \mu g^{-1}\ day^{-1}$)	0.95^{a}	0.15^{b}	0.15^{bb}	0.16^{bc}
K (maximum no. per plant)	1733.37^{a}	92.48^{b}	512.83^{c}	210.06^{d}
Tk (day)	38.20^{a}	18.18^{b}	30.70^{c}	24.00^{d}

Dissimilar alphabets with mean values in a row indicate significant differences by Tukey's multiple range test. GR = Growth rate; MRGR = Mean relative growth rate; K = Carrying capacity; Tk = Time taken to reach the 'K' level.

Growth parameters

The GR, MRGR, K and Tk found for the cotton clones were greater than those recorded for the clones from three other host plants (Table 2). The GR, K and Tk were the lowest in aphid clones from brinjal. K values varied from a low of 92.48 aphids per plant of brinjal to a high of about 1734 aphids per plant of cotton, but MRGR remained nearly the same on the three aphid clones from brinjal, chilli and arum plants and considerably higher at 0.95 $\mu g\ \mu g^{-1}\ day^{-1}$ in case of clones from cotton plants.

Esterase pattern

Number of esterase enzyme bands separated by electrophoresis varied in the clones of *A. gossypii* (Fig. 1, Table 3). Cotton clones were distinguished from the rest in possessing bands of higher mobilities (Est 6, 7, 9 and 11). Clones examined from cultivated varieties of brinjal (Br), chilli (Ch) and arum (Ar) showed bands of lower and higher mobilities. Five of these bands (Est 2, Est 3 and Est 7, Est 8 and Est 9) were found in all the clones. Brinjal clones possessed an exclusive band of slowest mobility (Est 1) whereas cotton clones possessed an exclusive band of highest mobility (Est 11). Arum clones could be distinguished from the rest in possessing an exclusive band at position Est 4. In general, bands of higher mobilities were dense and prominent and those of lower mobilities were diffuse and light, in particular bands at Est 5 position in clones from chilli and arum plants.

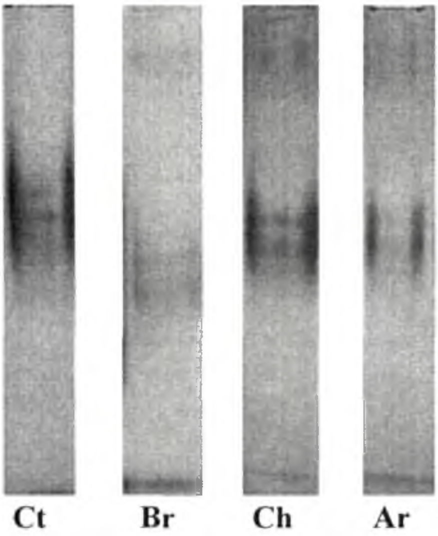

FIGURE 1. Isoenzymatic pattern of esterase of *Aphis gossypii* clones reared on cotton (Ct), brinjal (Br), chilli (Ch) and arum (Ar) plants

TABLE 3. Relative mobility of bands of esterase in the clones of *A. gossypii* reared on cultivated varieties of cotton, brinjal, chilli and arum

Bands	Relative mobility of bands in the aphid clones of host plants			
	Cotton	Brinjal	Chilli	Arum
Est-1	–	0.029	–	–
Est-2	–	0.071	0.071	0.079
Est-3	–	0.100	0.129	0.136
Est-4	–	–	–	0.164
Est-5	–	–	0.321	0.329
Est-6	0.372	–	–	0.407
Est-7	0.436	0.443	0.443	0.436
Est-8	–	0.471	0.486	0.471
Est-9	0.500	0.514	0.514	0.514
Est-10	0.551		0.557	0.564
Est-11	–	0.586	–	

DISCUSSION

The results of this study clearly show that *A. gossypii* clones from different host plants vary in their morphology, growth parameters and esterase enzymes. The body,

siphunculi, proboscis and antennae were longer and rate of increase was greater in *A. gossypii* reared on cotton plants than those reared on brinjal, chilli and arum plants. Aphids from chilli plants were the smallest and their rate of increase on this plant and those from brinjal and arum plants were found to be six-times lower than the aphids on cotton plants.

Esterase enzyme showed isozyme pattern in *A. gossypii* clones from the four host plants. Three bands, Est 7, 9 and 10, were present in all the clones but the other bands showed significant variations.

As this study was based on asexual aphids of *A. gossypii*, genetic variability as a possible cause of variations in populations reared on different host plants can not be implicated. In Japan, China and USA, some populations of *A. gossypii* show cyclical parthenogenesis consisting of one sexual generation followed by several asexual generations (Inaizumi, 1981; Ebert and Cartwright, 1997) and these aphids perform better on cotton, cucurbits and chrysanthemum than on other host plants with wide variations in their colonization success and rate of increase. These host-based relations have been attributed to genetic component due to variations in sexual populations from different plants (Guldemond *et al.*, 1994; Wool *et al.*, 1995). Given that there has been no reported occurrence of sexual reproduction in *A. gossypii* in India and other parts of South Asia, two factors could contribute to the observed variability in *A. gossypii* populations. The first factor seems to be the host plant specialization which could affect the distribution of genetic variability in aphid population (De Barro *et al.*, 1995). Using random amplified polymorphic DNA markers, it has been shown that populations of *A. gossypii* collected on plants from same family were multiclonal (Vanlerberghe-Masutti and Chavigny, 1998). The second factor that might contribute to the clonal diversity in *A. gossypii* could result from differential susceptibility to the toxic stimuli from pesticides. Aphids reared on different host plants are found to show different level of resistance (McKenzie and Cartwright, 1994). However, in the context of South Asia having more of diversified tropical flora and less of monoculture cropping pattern, host plant specialization factor seems to be the main basis of ecological and genetic divisions in *A. gossypii* populations. In conclusion, this study has provided information concerning diversity in asexual populations of *A. gossypii* on four host plants in NE India.

ACKNOWLEDGEMENTS

Authors are thankful to the Indian Council of Agricultural Research, New Delhi and Council of Scientific & Industrial Research, New Delhi for financial assistance. Prithwijyoti Bhwmik, SRF, assisted in the preparation of the manuscript.

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(Received 6 November 2006; accepted 15 May 2007)

ENTOMON **32(2)**: 97–102 (2007)
Article No. ent.32204

Biology and efficiency of the potential coccinellid predators of cowpea aphid, *Aphis craccivora* Koch. in cowpea

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ABSTRACT: The efficacy of the potential predators of the pea aphid *Aphis craccivora* infesting cowpea was assessed in laboratory. Among the major predators, the coccinellids, *Coccinella transversalis*, *Harmonia octomaculata* and *Menochilus sexmaculatus*, were found to be efficient ones since both the grubs and adults were predaceous and present in cowpea fields in all the seasons. Among these coccinellid predators, *H. octomaculata* had the shortest life cycle of 13.27 days followed by *C. transversalis* with 13.78 days and *M. sexmaculatus* with 18.7 days. A single grub of *C. transversalis* consumed a significantly high number of *A. craccivora* (251.8) compared to *H. octomaculata* consuming 198.22 and *M. sexmaculatus* consuming 127.6 aphids during its life time. Similarly per day consumptions of 30.77, 27.88 and 23.33 and a total consumption of 914, 842 and 734.1 *A. craccivora* were observed in the case of adult *C. transversalis*, *H. octomaculata* and *M. sexmaculatus*, respectively. Hence the most potential predator *C. transversalis* and the most predominant *M. sexmaculatus* could be promoted as biocontrol agents in the IPM schedule of grain cowpea.

KEYWORDS: feeding potential, *Coccinella transversalis*, *Menochilus sexmaculatus*, *Harmonia octomaculata*

INTRODUCTION

The cowpea crop is damaged intensively by a large number of insect pests at various stages of its growth especially during flowering and pod formation stages.To tackle these pests, farmers often resort to frequent and massive application of insecticides even in pod bearing stage which often results in serious residue hazards. Though there are many reports on pests and natural enemies of vegetable cowpea, the same is very meagre in dual-purpose cowpea varieties like Kanakamoni. Hence the

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TABLE 1. Biology of coccinellid predators of pea aphid *A. craccivora*

Predator	Duration in days							Total Life cycle in days
	Egg	1st instar	2nd instar	3rd instar	4th instar	pre pupa	pupa	
C. transversalis	2.71	1.20	1.33	2.67	3.00	1.20	1.67	13.78
M.sexmaculatus	2.10	1.20	2.80	3.60	3.80	1.80	3.40	18.70
H. octomaculata	2.20	1.50	1.83	2.36	2.36	1.20	1.82	13.27
CD (0.05)	0.207	0.159	0.148	0.205	NS	NS	0.148	1.25

present investigation was undertaken for assessing the efficacy of commonly occurring potential coccinellid predators in controlling the pea aphid *A. craccivora*.

MATERIALS AND METHODS

The potential coccinellid predators of cowpea aphid, *A. craccivora* viz. *C. transversalis*, *H. octomaculata* and *M. sexmaculatus* were collected from the cowpea fields of Onattukara Regional Agricultural Research Station, Kayamkulam and from the nearby farmers' fields. The predators were reared on aphids in the laboratory. Their efficacy was assessed in the following manner.

Adult beetles of *C. transversalis*, *H. octomaculata* and *M. sexmaculatus* collected from the field on glyricidia and cowpea infested with *A. craccivora*, were placed in glass troughs for egg laying. The eggs laid by adults were collected and ten freshly laid eggs of each coccinellid kept individually in glass vials and covered with muslin cloth. The incubation period was observed. On hatching, each grub was introduced into a colony of known number of *A. craccivora* adults placed on cowpea grown in an ice cream cup wrapped at the base by wet blotting paper. Ten replications were maintained and the duration of each instar *viz.*, first, second, third and fourth were recorded and the mean were worked out.

The feeding potential in terms of the number of aphids consumed daily by the second, third and fourth instar grubs were recorded and the mean worked out. The adult beetles required for the feeding experiment were reared from the pupae collected from cowpea fields. The mean consumption of *A. craccivora* by adult beetles was also recorded.

RESULTS

The first instar grubs of coccinellids were not found feeding on the aphids. The third and fourth instar grubs and adults of all the three predators were voracious feeders on *A. craccivora*. The grubs and adults of coccinellids were found to eat only live aphids.

The mean duration of the developmental stages of *C. transversalis*, *H. octomaculata* and *M. sexmaculatus* are given in Table 1.

The total life cycle of *M. sexmaculatus* was observed to be 18.7 days which

TABLE 2. Feeding potential of the grubs of coccinellid predators of pea aphid

Predator	Mean No. of aphids consumed by different instars			Total consumption
	2	3	4	
C. transversalis	24.70	89.66	137.44	251.80
H. octomaculata	17.64	74.18	106.4	198.22
M. sexmaculatus	10.70	35.90	81.00	127.60
CD (0.05)	–	–	–	29.30

had significant superiority over the other two species viz. *C. transversalis* and *H. octomaculata* with 13.78 and 13.27 days respectively.

The mean incubation period was the minimum for *M. sexmaculatus* (2.10 days) which was on par with that of *H. octomaculata* (2.20) but significantly shorter than *C. transversalis* (2.71 days).

The duration of the first instar grub was significantly longer for *H. octomaculata*. The second and third instar larval periods were the longest for *M. sexmaculatus* being 2.8 and 3.6 days respectively. The duration of fourth instar grub and prepupal period though not significant were also the longest for *M. sexmaculatus*. The pupal period was significantly longer (3.4 days) for *M. sexmaculatus* than the other two coccinellids. In general the total larval period (11.4 days) and mean longivity (31.5 days) were maximum for *M. sexmaculatus* which is a desirable character for a predator. The adult mean longevity of *C. transversalis* and *H. octomaculata* were 29.7 and 30.1 days respectively.

The mean number of aphids consumed by second third and fourth instar grubs of *C. transversalis* were 24.7, 89.66 and 137.44 respectively (Table 2). Thus a single grub consumed a mean number of 251.8 aphids during its life time which was significantly superior over *H. octomaculata* with 198.22 and *M. sexmaculatus* with 127.6 aphids. The second, third and fourth instar grubs of *H. octomaculata* consumed a mean number of 17.64 , 74.18 and 106.4 aphids whereas *M. sexmaculatus* consumed 10.7, 35.9 and 81 aphids respectively. So among the coccinellid grubs, *C. transversalis* was the most efficient one which consumed maximum number of aphids during its life time.

The adults of the coccinellid *C. transversalis* during its life time consumed a mean number of 914 adult aphids within a period of five weeks which was statistically superior over *H. octomaculata* which consumed 842 aphids in five weeks time and *M. sexmaculatus* with 734.1 aphids in 7 weeks time (Table 3). Hence in the case of efficiency of adults also *C. transversalis* ranked first.

DISCUSSION

The potential predators of pea aphid *A. craccivora* identified were *C. transversalis*, *H. octomaculata* and *M. sexmaculatus*. The biology and feeding potential of these major predators were studied and discussed.

The non feeding nature of the first instar grub observed in the present study may

TABLE 3. Feeding potential of the adult coccinellid predators of pea aphid

Predator	Mean No. of aphids consumed (days)							Total consumption
	1	2	3	4	5	6	7	
C. transversalis	122.0	185.0	200.5	268.0	138.5	–	–	914.0
H. octomaculata	110.0	158.2	178.5	240.0	155.3	–	–	842.0
M. sexmaculatus	110.0	148.0	152.4	160.8	102.0	42.0	18.9	734.1
CD (0.05)	–	–	–	–	–	–	–	30.98

be attributed to the cannibalistic behaviour of the first instar on unfertile eggs of the same batch as reported by Dixon (1959) and Murdoch (1971). The third and fourth instar grubs were found to consume an appreciable number of nymphs and adults of *A. craccivora* compared to second instar. This might be due to the increased age and capture efficiency by a process of learning (Murdoch, 1971).

Among the three species, *M. sexmaculatus* has the longest life cycle of 18.70 ± 0.91 days. Nandakumar (1999) observed the total duration of *M. sexmaculatus* from egg to adult as 15.35 days. Begal and Trehan (1949) reported that the duration and even the number of instars vary with season.

The mean incubation period was significantly shorter for *M. sexmaculatus* (2.10 days) and the longest for *C. transversalis* with 2.71 days. These findings are in agreement with the findings of Hagen (1962) and Verma *et al.* (1993). On the contrary Rai and Singh (2001) reported a higher incubation period of 8.7 days in January than in February (8.1) and March (5.8), a phenomenon attributed to the low temperature prevailing in January.

The mean longevity of adults were 29.7, 30.1 and 31.5 days, respectively, for *C. transversalis*, *H. octomaculata* and *M. sexmaculatus*. Rai and Singh (2001) also reported that the larval duration and longevity of adults were also higher in January than in February and March.

Among the three major predators, the grubs of *C. transversalis* consumed the maximum number of 251.8 *A. craccivora*. A wide variation in the consumption of aphids by a single grub of *C. transversalis* (401–736) was observed by Debaraj and Singh (1990) whereas Begal and Trehan (1949) observed a variation of 106.29 to 420.00 in the consumption of aphids by *Coccinella* sp. The percentage consumption by the second, third and fourth instar grubs of *C. transversalis* were 9.81, 35.61 and 54.58 aphids respectively with a mean per day consumption of 31.48. This was followed by *H. octomaculata* with feeding potential of 198.22 aphids with an average per day consumption of 24.78. Joshi *et al.* (1999) observed *H. octomaculata* to be feeding on *A. craccivora* infesting *Casia auriculata* and *Crotalaria mucronata*. With regard to *M. sexmaculatus*, the mean total consumption of *A. craccivora* by a single grub was 127.6, the per day consumption being 10.63 aphids. Lokhandae and Mohan (1990) and Rani (1995) earlier reported that a single grub of *M. sexmaculatus* consumed 73.52 and 84 aphids respectively whereas Begal and Trehan (1949) observed a high rate of 303 aphids per grub.

A similar trend was observed in the case of adults of these coccinellids also. A per day consumption of 30.77, 27.88 and 23.3 and a total consumption 914, 842 and 734.1 *A. craccivora* was observed in the case of *C. transversalis*, *H. octomaculata* and *M. sexmaculatus*, respectively. Das and Premsagar (2001) observed a per day consumption of *A. craccivora* by *C. septempunctata* to be 26.97 whereas Joshy *et al.* (1999) observed it to be 40.6. A wide variation in the per day consumption of aphids (17 to 57) was reported by several workers (Devi, 1967; Haque and Islam, 1978).

From this study it is evident that the coccinellid predator, *C. transversalis* is the most efficient one. Though the total life cycle, especially larval period and adult longevity was found to be the maximum for *M. sexmaculatus*, the feeding potential of both the grubs and adults of this coccinellid was significantly lower than the other two. Though the number of aphids consumed by the grubs and adults of *H. octomaculata* was significantly higher than *M. sexmaculatus*, this coccinellid was rare in the grain cowpea fields in summer season. Hence, considering the high potential of *C. transversalis* and longer larval period and adult longevity of *M. sexmaculatus*, these two coccinellids could be promoted as biological control agents in the IPM technology for grain cowpea.

ACKNOWLEDGEMENTS

This paper forms a part of the Ph.D thesis submitted to the Kerala Agricultural University (2003) by the senior author. We are thankful to the Dean, College of Agriculture, Vellayani and the Project Director & Head, Onattukara Regional Agricultural Research Station, Kayamkulam for the facilities provided for the study.

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(Received 6 February 2007; accepted 15 May 2007)

ENTOMON **32(2)**: 103–109 (2007)
Article No. ent.32205

Scarabaeid beetles of Kullu valley, Himachal Pradesh

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ABSTRACT: Light trap studies were carried out at Bajaura (1100 m amsl), Katrain (1500 m amsl) and Targali (1150 m amsl) of Kullu valley of Himachal Pradesh to identify the species of scarabaeid beetles present in the three regions. In total 29, 19 and 18 species of the beetles were collected at Bajaura, Katrain and Targali, respectively. Out of these, *Anomala rufiventris* Redt., *A. lineatipennis* Blanch., *Melolontha nepalensis* Blanch., *M. furcicauda* Ancey, *Melolontha* sp., *Adoretus simplex, A. duvauceli, Brahmina flavoserica* (Bost), *B. crinicollis* Burm., *Xylotrupes gideon (Linn.)* , *Maladera* sp., *Meriserica* sp., *Catharsius* sp., *Onthophagus* sp., *Macronota* sp., *Popillia maclellandi* Hope, *Mimela* sp. and *Leucopholis* sp. are new records from Kullu valley. *Melolontha nepalensis* Blanch., *Adoretus simplex, A. duvauceli, Brahmina flavoserica* (Bost), *Meriserica sp., Catharsius* sp., *Onthophagus* sp. and *Macronota* sp., have been collected for the first time from Himachal Pradesh. Emergence of beetles peaked in the last week of June, first or second week of July at all the three localities. Minimum temperature had significant positive correlation with the emergence of beetles.

KEYWORDS: scarabaeid beetles, white grubs, new records, *Anomala, Melolontha, Brahmina, Adoretus, Xylotrupes, Holotrichia, Maladera*

INTRODUCTION

Scarabaeid beetles (Coleoptera : Scarabaeidae) are polyphagous agricultural pests of great importance. As adults they defoliate trees and in larval stage (popularly known as white grubs or root grubs) inflict heavy damage to many field crops in Himachal Pradesh (Chandla *et al.*, 1988; Misra, 1992; Kumar *et al.*, 2005). Light trap has been extensively used for monitoring the beetle population in different parts of the state (Chandel *et al.*, 1994; Kumar *et al.*, 1996). The present status of the pest in two geographically and climatically distinct locations viz., Katrain and Targali, and an already surveyed area, Bajaura, of Kullu district of Himachal Pradesh was assessed using light trap collection and the results are presented in this paper.

MATERIALS AND METHODS

The investigations were undertaken at three locations viz., Bajaura, Katrain and Targali. Katrain and Targali locations are 40 km and 30 km in north and south direction, respectively, from Bajaura and 70 km apart from each other. A light trap made of galvanized tin sheet fitted with 160 watts mercury electric bulb as light source was installed in the field at about 2.5 m height on an iron pole at each location. Beetles of different species trapped in the polythene bag tied to the stem of a funnel fitted to the bottom of the tin sheet of the trap were collected and killed in benzene, counted species-wise and preserved. Observations on the light trap catches of the beetles were started in the second week of March and continued till September end. These observations were recorded for three nights in each week and data were pooled week wise as well as month wise. Beetles were got identified from Directorate of All India Network Project on White Grubs, Department of Entomology, University of Agricultural Sciences, Bangalore, Karnataka.

Data on weather parameters viz., temperature, relative humidity and rainfall were obtained from meteorological observatory near Bajaura and Katrain. Simple correlation of these parameters with beetle catch was done through path co-efficient analysis suggested by Dewey and Lu (1959).

RESULTS AND DISCUSSION

Species composition and new records

Data (Table 1) showed that 29, 19 and 18 species of scarabaeid beetles were collected in the light trap at Bajaura, Katrain and Targali, respectively. Among these, *Holotrichia longipennis* Blanchard, *Anomala dimidiata* (Hope), *A. rufiventris* Redt., *Melolontha furcicauda* Ancey, *Adoretus simplex*, *Adoretus* spp., *Brahmina coriacea* (Hope), *B. flavoserica* (Bost), *Xylotrupes gideon* (Linn.) and *Maladera* sp. were recorded from all the three locations. *A. lineatipennis* Blanch., *A. polita, Melolontha* sp., *B. crinicollis* Burm., *Onthophagus* sp., *Macronota* sp., *Mimela* sp. and *Leucopholis* sp. were recorded only at Bajaura while *Popillia maclellandi* Hope and *Melolontha nepalensis* Blanchard only at Katrain and Targali, respectively. *Adoretus duvauceli, Maladera insanibilis* (Blanchard) and *Catharsius* sp. were not recorded at Targali and *Phyllognathus dionysius* Fab. at Katrain.

Anomala rufiventris Redt., *A. lineatipennis, Melolontha nepalensis* Blanch., *M. furcicauda, Melolontha* sp., *Adoretus simplex, A. duvauceli, B. flavoserica, B. crinicollis, X. gideon, Maladera* sp., *Meriserica* sp., *Catharsius* sp., *Onthophagus* sp., *Macronota* sp., *Popillia maclellandi, Mimela* sp. and *Leucopholis* sp. are the new records of scarabaeid beetles from Kullu valley and *M. nepalensis, A. simplex, A. duvauceli, B. flavoserica, Meriserica* sp., *Catharsius* sp., *Onthophagus* sp., *Macronota* sp., have been collected for the first time from Himachal Pradesh.

TABLE 1. Species of white grub beetles collected in the light trap at three locations during 2002 to 2003

	Prevalence at location		
Species	Bajaura	Katrain	Targali
Melolonthinae			
Holotrichia longipennis Blanchard	+	+	+
Melolontha sp.	+	−	−
Melolontha nepalensis Bl.	−	−	+
Melolontha furcicauda Ancey	+	+	+
Brahmina coriacea (Hope)	+	+	+
B. flavoserica (Bost)	+	+	+
B. crinicollis Burm.	+	−	−
Maladera insanibilis (Blanch).	+	+	−
Maladera sp.	+	+	+
Meriserica sp.	+	−	−
Leucopholis sp.	+	−	−
Rutelinae			
Anomala dimidiata (Hope)	+	+	+
Anomala rufiventris Redt.	+	+	+
A. lineatipennis Blanch.	+	−	−
A. polita Blanch.	+	−	−
Adoretus simplex	+	+	+
Adoretus duvauceli	+	+	−
Adoretus spp.	+	+	+
Popillia maclellandi Hope	−	+	−
Mimela sp.	+	−	−
Dynastinae			
Xylotrupes gideon (Linn.)	+	+	+
Phyllognathus dionysius Fab.	+	−	+
Cetoninae			
Macronota sp.	+	−	
Catharsius sp.	+	+	−
Onthophagus sp.	+	−	−
Others			
Unidentified sp.	6	5	6
Total number of species at different locations	29	19	18

+ Present, − Absent

Relative abundance

Data (Table 2) showed that at Bajaura, during 2002 and 2003, *Adoretus* spp. (including *A. simplex*) were predominant. These were followed by *Maladera* spp., *B. coriacea*, *A. dimidiata* and *H. longipennis* during 2002 and by *Maladera* spp. and *A. dimidiata* during 2003. 'Other beetles' which included *A. rufiventris*, *P. dionysius*,

TABLE 2. Proportion of dominant species of white grub beetles in the light trap at different locations during 2002 and 2003

Species	Percentage of different species out of total catch					
	Bajaura		Katrain		Targali	
	2002	2003	2002	2003	2002	2003
Holotrichia longipennis Blanchard	10.66	4.22	17.85	14.23	18.43	12.85
Brahmina coriacea (Hope)	12.87	4.00	26.87	15.01	20.55	14.26
Maladera spp.	17.37	26.67	12.13	16.57	12.08	10.74
Anomala dimidiata (Hope)	12.55	10.00	4.33	6.63	5.72	12.68
Adoretus simplex	10.21	16.22	10.57	11.89	14.83	12.32
Adoretus spp.	20.73	17.67	18.20	16.57	8.89	12.85
Xylotrupes gideon (Linn.)	8.76	4.89	0.52	1.75	2.54	4.93
Other Beetles	6.86	16.33	9.53	17.35	16.95	19.37

TABLE 3. Month wise proportion of beetles of white grubs collected in the light trap at different locations during 2002 and 2003

Month	Percentage of total catch					
	Bajaura		Katrain		Targali	
	2002	2003	2002	2003	2002	2003
March	1.17	1.33	0.00	2.14	0.00	0.00
April	2.48	1.67	6.76	10.72	0.42	0.35
May	2.34	1.89	11.09	12.28	10.59	10.74
June	18.25	16.22	42.29	33.14	38.14	36.80
July	45.84	56.78	34.49	36.26	36.02	39.96
August	23.06	21.22	4.85	5.07	14.41	11.80
September	6.86	0.89	0.52	0.39	0.42	0.35

A. lineatipennis, *Anomala* sp. and some unidentified species together constituted 16.33 per cent of the total catch during 2003 and only 6.86 per cent during 2002.

At Katrain and Targali, *Adoretus* spp. (including *A. simplex*) were predominant during both the years (2002 and 2003) of study. At Katrain, these were followed by *B. coriacea*, *H. longipennis* and *Maladera* spp. However, their proportion varied slightly during the two years. 'Other beetles' which included *A. rufiventris*, *B. flavoserica*, *M. furcicauda*, *Catharsius* sp, *P. maclellandi* and some unidentified species constituted 9.53 and 17.35 per cent of total catch during the two years respectively.

At Targali, *Adoretus* spp. were followed by *B. coriacea*, *H. longipennis* and *Maladera* spp. during 2002 and by *B. coriacea*, *H. longipennis* and *A. dimidiata* during 2003. 'Other beetles' which included *A. rufiventris*, *M. furcicauda*, *M. nepalensis*, *B. flavoserica*, *P. dionysius* and some unidentified species together constituted 16.95 and 19.37 per cent of total catch during the two respective years.

TABLE 4. Pattern of emergence of dominant species of white grubs at three locations during 2002 and 2003

Month/ week	Total No. of beetles collected per trap per week[a]																																															
	H. longipennis						*A. dimidiata*						*Maladera* spp						*Adoretus simplex*						*Adoretus* spp.						*B. coriacea*						*X. gideon*						Other Beetles					
	B		K		T		B		K		T		B		K		T		B		K		T		B		K		T		B		K		T		B		K		T		B		K		T	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
March II																																												12		5		
III																									5																					3		
IV																									3																			12		3		
April I																									6		3	2																		5		
II																									7		8	6																6		14		
III																									3		17	9		2								3						2		9		
IV											2														1		11	2																4		8		
May I											4	5													8	1	6	10		2			6	4				1										
II											7	3													2		6	8	2				15	8	2			1			3					2		2
III												6						1				2						6	5	3	2		14	4	5	3	1	2			1	4		2				9
IV				2	6	6						7				4		2								4		7	3	4	3		17	4	9	3		3						3		2	3	1
June I	4		9	4	8	9	1			2		4			1	3	2	4				2			3	6	3	2	3	3	4		23	7	11	8		3					1	3		6	6	5
II	6		14	7	12	7	2	2				2	1		1	2	4	4	1		7	7	1		3	8	1	2	6	9	4	2	9	9	7	8		1		1	1	3	1	3	3	2	6	12
III	9		17	7	8	7	5	6		3		2	6	9	6	11	8	9		6	13	7			9	1	9	9	4	8	6	2	12	6	12	11	4	2		2		3		11	8	5	14	13
IV	11	2	13	13	14	10	8	19	2	7		6	8	27	24	16	13	5	3	11	18	10	3		16	4	15	8	7	14	3	9	20	8	15	19	6	1	1					8	15	2	13	11
July I	15	7	8	15	7	7	10	8	3	11	3	11	21	61	20	20	9	9	7	9	7	6	7		13	24	8	3	6	11	7	5	11	10	8	10	9	2		1		5	12	11	10	3	7	15
II	8	4	6	8	5	4	7	18	3	5	1	7	14	55	13	16	9	9	13	36	7	9	13		18	32	6	3	1	6	17	2	9	7	8	10	3		2	3	3	3	7	30	8	5	6	14
III	5	2	5	4	6	4	9	5	6	2	5	10	12	34	5	7	7	11	10	19	9	8	10		11	20	8	5	4	3	13	1	9	4	13	4	4	7		1	2	2	8	10	4	2	8	8
IV	4	10	9	3	7	3	6	14	8	3	3	7	13	18		4	2	3	4	17		8	4		11	22	3	2	1	4	11	9	8	4	2	2	4			1			8	19	4	3	3	6
Aug. I	6	7	7	2	4	5	9	10	3	1	2	2	8	9		2	2	1	9	18		2	9		5	16	1	1		4	8	2	2	2	4	2	3	13				4	4	9	3	3		4
II	4	1	3	2	7	6	5						9	14			1	3	11	14			11		3	13					4				1	1	4	5				2	4	3		1	3	3
III		2	4	3	2	3	7	7					4	6					8	8			8		7	6					3	2					6					2	2	6		6	5	3
IV	1	2	5	1	1	2	3	1					3	7					6	6			2		4	2					2	1					10							1			6	4
Sept. I		1	3	2			4						3						2	2			2								1	1					6							4				2
II–IV							10						17																																			

B, Bajaura; K, Katrain; T, Targali

[a] Blank represents zero

* 1, data for 2002; 2, data for 2003

Predominance of different species in different localities during different years can be attributed to the difference in the meteorological factors in addition to species pool prevalent in that area. 'Other beetles' which consisted of different species and constituted different proportion in different localities showed that these scarabaeid beetles are either of lesser significance in these localities or exhibit poor photo-positive response.

Period of activity

Total catch of all the species was maximum in June/July in both the years (Table 3). It agrees with earlier reports (Gupta *et al.*, 1977; Chandel *et al.*, 1994; Kumar *et al.*, 1996).

The correlation studies revealed a significant positive association with minimum temperature. When minimum temperature started increasing in April with showers of rainfall, beetles started appearing and their catch peaked in June/July when the average minimum temperature varied from 17 °C to 21 °C at Katrain and 16 °C to 22 °C at Bajaura during different years.

Pattern of beetle emergence

The temporal pattern of emergence of the dominant whitegrub beetles at the three locations durin gthe year 2002 and 2003 is shown in Table 4.

At Bajaura, during during 2002, *Adoretus* spp. were the first to appear in the light trap in the 3rd week of March. *Brahmina coriacea* and *X. gideon* were first caught in the 3rd week of May. Most of the species including *H. longipennis* and *A. dimidiata* started appearing in the first week of June. During 2003, some unidentified species were the first to appear in the light trap in the last week of March. *X. gideon* was first caught in the 3rd week of April. *Adoretus* spp. started appearing in the first week of May; *P. dionysius* in the first week of June; *Anomala dimidiata* and *B. coriacea* in the 2nd week of June and *Maladera* spp in the 3rd week of June. *H. longipennis* was first caught in the light trap as late as in the last week of June.

At Katrain, during both the years of study, *Adoretus* spp. were the first to be caught in the light trap in the first week of April and *B. coriacea* in the first week of May. However, *H. longipennis* and *Maladera* spp. were caught first in the first week of June during 2002 while in the last week of May during 2003. During 2003, some unidentified beetles started appearing in the 2nd week of March and *A. dimidiata* in the 1st week of June.

At Targali, during 2002, *A. dimidiata* was the first species to be caught in the light trap in the last week of April; *B. coriacea*, X. gideon and *Adoretus* spp. were first caught in the light trap in the 2nd week of May and *H. longipennis* in the last week of May. During 2003, *Adoretus* spp. were the first to appear in the light trap in the 3rd week of April. *Anomala dimidiata* were first trapped in the first week of May; *P. dionysius* in the 2nd week of May; *B. coriacea* and *X. gideon* in the 3rd week of May; *H. longipennis* and *A. rufiventris* in the last week of May.

Temporal differences observed in the emergence of beetles during different years can be attributed to the difference in climatic conditions during these years.

ACKNOWLEDGEMENTS

Authors thankfully acknowledge the ICAR, New Delhi for financial assistance in the form of NATP sub-project. Authors are also thankful to Dr. T. M. Musthak Ali, Entomologist and PI, AINP on Whitegrubs, Department of Entomology, UAS, GKVK, Bangalore (Karnataka state) for the identification of specimens.

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(Received 16 April 2007; accepted 15 May 2007)

ENTOMON 32(2): 111–117 (2007)
Article No. ent.32206

Influence of baseline variation on the biological performance of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in South India

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ABSTRACT: Populations of *H. armigera* were collected from cotton fields of three major cotton growing states in South India, *viz.*, Andhra Pradesh (Guntur and Warangal), Karnataka (Raichur and Dharwad) and Tamil Nadu (Attur and Coimbatore). The study showed that the total, egg, larval, and pupal developmental time, adult longevity and malformed adult emergence (%) were significantly lower in Guntur population and highest in Coimbatore population whereas the developmental indices, adult emergence, fecundity, fertility and oviposition period were higher in Guntur population and lowest in Coimbatore populations. The growth and food utilization were significantly varied among the populations. Consumption index, growth rate, efficiency of conversion of ingested food and efficiency of conversion of digested food were highest in Guntur population and lowest in Coimbatore population. The results highlight the need for development of location specific pest management strategies.

KEYWORDS: baseline variation, biological performance, *H. armigera*

INTRODUCTION

The cotton bollworm (*Helicoverpa armigera* Hub.) is an important polyphagous pest and causes 14 to 56% damage in cotton alone. The variation in crop loss among locations is attributed to differences in populations of *H. armigera* and their susceptibility to chemical pesticides (Butter and Brar, 1999). This is a facultative migratory insect and can fly over long distance (Wu and Guo, 1995). This migratory behaviour may create chance for inter-crossing among the populations over the locations, which aggravate the variations in susceptibility to insecticides and their level of resistance (Armes *et al.*, 1996). Variations in the biological performance of *H. armigera* over the locations may increase the possibility of rate of development of

resistance to *Bt* cotton. The present investigation was carried out to establish a base line data on the biological performance of *H. armigera* in cotton growing regions of South India which may give a way for further investigation on genetic diversity of *H. armigera* to formulate location specific pest management strategies.

MATERIALS AND METHODS

RCH 2 hybrid of cotton was raised in pots and maintained in green house when the daily temperature ranged between a minimum of 20 to 24 °C and a maximum of 30 to 34 °C. Planting of seeds was done at weekly intervals to ensure continuous availability of quality leaves for rearing the test insects.

Third instar larvae of *H. armigera* were collected from cotton fields from six locations, two each from the cotton growing states of South India *viz.*, Guntur and Warangal (Andhra Pradesh), Dharwad and Raichur (Karnataka); Coimbatore and Attur (Tamil Nadu). The larvae were reared till pupation on a modified semi synthetic diet (Patel *et al.*, 1968). Adults emerging from each lot were transferred to separate glass jar, containing 10% honey as food, for egg laying. Emerging larvae were separately maintained in the laboratory on cotton leaves and the larvae collected from second generation were used for different experiments.

Variations in biology of *H. armigera* populations

Data on the duration of development of larval stages, adult longevity, adult emergence, larval and pupal weights were recorded by daily observation of different rearings. The larva were weighed individually (ten individuals/replication) with a digital balance (Sartorius New York) on 3rd, 7th and 11th days and pupae at 3rd day after pupation. Male and female pupae were weighed separately and developmental index following Butter and Brar (1999) was calculated. Fecundity, fertility and oviposition period were studied by rearing the adults emerging from respective populations in round plastic containers with two pairs/container for each replication.

Determination of growth and nutritional indices

The pre-weighed leaves were placed in polypots and subsequently four hours pre-starved and pre-weighed third instar larvae were released. Observations on quantity of food consumed, unconsumed, excreta voided and the weight gained by the larvae from third instar to fourth instar were recorded. From the data, Consumption index (CI), Growth rate (GR), Efficiency of conversion of ingested food (ECI), Approximate digestibility (AD) and Efficiency of conversion of digested food (ECD) were calculated following Waldbauer (1964).

RESULTS

Variations in biology of *H. armigera* populations

The data presented in Table 1 and 2 show the variation in biological performance of different populations of *H. armigera* in South India. Table 1 explains the variation

in developmental durations and developmental index of different *H. armigera* population. Thus, the total egg, larval, pupal developmental time and adult longevity (from egg laying to egg emergence, egg emergence to pupation, pupation to adult emergence and from adult emergence to adult mortality) was significantly lower in Guntur (3.13, 11.88, 8.50 and 10.50 days) and Warangal, (3.13, 12.38, 8.88 and 10.75 days) than Attur (3.13, 14.63, 9.38 and 8.43 days), Raichur (3.13, 13.25, 9.00 and 10.45 days), Dharwad (3.13, 13.75, 9.25 and 9.75 days) and Coimbatore (3.13, 15.00, 9.25 and 8.88 days) population respectively. The total developmental process of *H. armigera* (from egg laying to adult mortality) was significantly higher in Coimbatore (36.51 days) than in Dharwad, Raichur, Attur, Warangal and Guntur population (36.13, 35.83, 35.69, 35.14 and 34.01 days). The developmental index calculated in different population clearly showed that Guntur population has increased fitness. The lowest developmental index was influenced by extended larval period coupled with lowest per cent adult emergence. The developmental index of larvae of Attur, Coimbatore, Guntur, Warangal, Raichur and Dharwad were 2.23, 2.05, 2.49, 2.35, 2.28 and 2.19 respectively.

Adult emergence, healthy and malformed adult emergence rate of different populations of *H. armigera* varied from 79 to 84, 82 to 91 and 8 to 18%, respectively, among the locations. Lowest healthy adult emergence (82.35%) and highest malformed adults (17.65 %) were observed in Coimbatore population. Similarly, highest healthy adult emergence (91.87%) and lowest malformed adults (8.13%) were observed in Guntur population. Significant difference was observed in reproductive behaviour of different populations of *H. armigera* on cotton. The lowest number of eggs (532/ pair) was observed in Coimbatore population and higher egg laying (873/ pair) was recorded in Guntur population. The fertility of the eggs deposited by the moths varied with locations. The percentage of fertility was higher in Guntur (69.93) than in Warangal, Raichur, Dharwad, Attur and Coimbatore (67.06, 61.10, 58.95, 54.84 and 50.58, respectively). The duration of fecundity/oviposition was lower in Coimbatore and Attur population (5.4 and 5.9 days). But it was higher in Guntur and Warangal (7.9 and 7.5 days) than in Raichur and Dharwad (6.9 and 6.7 days) (Table 1).

The data presented in Table 2 indicate there was no significant difference in larval weight gain pattern of *H. armigera* among populations. The results of the study indicate that there was a significant variation on the weight of pupae. Increased female pupal weight was observed in Guntur population (293.60 gm/pupa) than in Warangal, Raichur, Dharwad, Attur and Coimbatore (286.58, 280.34, 275.87, 270.22 and 266.77 gm/pupa). Table 2 also shows that the growth and food utilization was significantly varied among the populations. Consumption index, growth rate, efficiency of conversion of ingested food and efficiency of conversion of digested food were highest in Guntur population (2.02, 0.34, 10.80 and 84.29, respectively) and lowest in Coimbatore population (1.47, 0.23, 4.23 8 and 61.85, respectively). Similarly, the approximate digestibility was lowest in Guntur population (40.53) and highest in Coimbatore population (48.17).

TABLE 1. Variations in the biological parameters of *H. armigera* collected from different cotton belts of South India*

Location	Duration of different life stages (Days) Egg	Larva	Pupa	Adult	Total	Index	Fecundity Egg/Female	Fertility (%)	% of moth emergence	Healthy adults (%)	Malformed adults (%)
Attur	3.13^{a}	14.63^{a}	9.38^{a}	8.43^{a}	35.69	2.23	598.25^{b}	54.84^{c}	79.41^{c}	85.18^{c}	14.82^{b}
Coimbatore	3.13^{a}	15.00^{a}	9.25^{a}	8.88^{a}	36.51	2.05	532.50^{a}	50.80^{d}	74.76^{d}	82.40^{d}	17.65^{a}
Guntur	3.13^{a}	11.88^{c}	8.50^{b}	10.50^{b}	35.01	2.49	873.25^{f}	69.93^{a}	84.81^{a}	91.87^{a}	08.13^{d}
Warangal	3.13^{a}	12.38^{c}	8.88^{b}	10.75^{b}	35.14	2.35	753.00^{e}	67.06^{a}	82.71^{ab}	88.81^{b}	11.19^{c}
Raichur	3.13^{a}	13.25^{b}	9.00^{ab}	10.45^{b}	35.83	2.28	689.25^{d}	61.10^{b}	81.62^{bc}	86.42^{c}	13.58^{b}
Dharwad	3.13^{a}	13.75^{b}	9.25^{a}	9.75^{b}	36.13	2.19	646.00^{c}	58.95^{b}	79.41^{c}	86.25^{c}	13.75^{b}

* Means of 20 observations; means followed by different letters within a column indicate significant differences ($P = 0.01$: LSD)

TABLE 2. Growth parameters in the development of larval/ pupal stages of *H. armigera* collected from different cotton belts of South India*

Location	Weight of larva/ pupa (mg)			Consumption index	Growth rate	Conversion of ingested food index	Conversion of digested food index	Approximate digestibility (%)
	11th day of larva	Male pupa	Female pupa					
Attur	318.47^{a}	269.49^{a}	270.22^{f}	1.47^{d}	0.24^{c}	4.75^{e}	47.42^{a}	66.24^{de}
Coimbatore	312.91^{a}	262.56^{a}	266.77^{e}	1.37^{e}	0.23^{c}	4.23^{f}	48.17^{a}	61.85^{e}
Guntur	338.09^{a}	262.97^{a}	293.60^{a}	2.02^{a}	0.34^{a}	10.80^{a}	40.53^{c}	84.29^{a}
Warangal	334.26^{a}	257.38^{a}	286.58^{b}	1.62^{c}	0.29^{b}	6.73^{c}	44.79^{b}	71.38^{bc}
Raichur	326.27^{a}	249.96^{b}	280.34^{d}	1.75^{b}	0.33^{a}	8.09^{b}	43.54^{b}	75.18^{b}
Dharwad	323.13^{a}	246.18^{c}	275.87^{c}	1.54^{cd}	0.27^{b}	5.28^{d}	46.31^{ab}	69.76^{cd}

* Means of 20 observations; means followed by different letters within a column indicate significant differences ($P = 0.01$: LSD)

DISCUSSION

The present study shows the geographical variation in development and reproductive behaviour of *H. armigera*. Growth and development of *H. armigera* showed significant variation among the populations. Across the locations, variations were found on total developmental duration *viz*., egg, larval, pupal and adult durations; and similar results were observed in *Helicoverpa armigera* in India (Butter and Brar, 1999). In the present study, there was no significant difference found in larval weight gain pattern of *H. armigera* populations. On the contrary, the pupal weight (male and female) showed significant variation and the results are in agreement with the findings of Butter and Brar (1999).

Variation in the reproductive capacity of *H. armigera* moths was seen over the locations. There was definite difference in adult emergence, healthy adults, malformed adult emergence, fecundity; fertility and fecundity period was observed when the yield of reproduction behaviour was compared. The present results thus agree with the earlier reports of Butter and Brar (1999), Patel and Talati (1987) and Tripathi and Singh (1993).

The present study also shows the baseline variation in consumption index, growth rate, efficiency of conversion of ingested food, approximate digestibility and efficiency of conversion of digested food of *H. armigera* populations from different geographic areas on the same host plant. Studies conducted by Butter and Brar (1999) revealed that the *H. armigera* population within the state of Punjab in India showed significant variation in some of the growth and food utilization indices. Studies conducted by Fakrudin *et al.* (2004) on the genetic variation of cotton bollworm, *Helicoverpa armigera* in South Indian cotton ecosystem suggested that the topographical barriers due to weather and environmental factors and temporal barriers due to cropping pattern might play a key role in isolating some population, resulting in high amount of genetic variability among the population. Further studies on genetic variation at molecular level will lead to better understanding and developing a management strategy against *H. armigera*.

ACKNOWLEDGEMENTS

We thank Department of Entomology, TNAU, Coimbatore and Department of Science and Technology, SERC, Fast Track YS Scheme for providing basic infrastructural facilities and fellowship to the Young Scientist (M.K.). Thanks are also due to Dr. B. Rosaiah, Associate Director of Research, Dr. Hariprasad Rao, Principal Scientist, ANGRAU, Lam farm, Guntur, Andhra Pradesh for assisting in larval collections and Dr. B. V. Patil, Associate Director of Research, Regional Research Station, Raichur for Karnataka collection.

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(Received 26 April 2007; accepted 15 May 2007)

ENTOMON **32(2)**: 119–127 (2007)
Article No. ent.32207

Butterflies in the Great Himalayan Conservation Landscape in Himachal Pradesh, Western Himalaya

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ABSTRACT: Seventy five species of butterflies belonging to 48 genera and five families were documented from different forest types and watershed in the Great Himalayan Conservation Landscape area of Himachal Pradesh. The butterfly composition (richness and diversity) was significantly higher in broad leaved forest compared to other forested habitats. Sub-alpine habitat had the most dissimilar butterfly species. The richness pattern also showed a positive trend with an increase in altitudinal gradient.

KEYWORDS: butterfly diversity, Himachal Pradesh, India

INTRODUCTION

The butterfly fauna of Indian sub-continent have been mainly studied by Talbot (1939), Wynter-Blyth (1957), D'Abrera (1982, 1985), Mani (1986), Haribal (1992), and Kunte (2000). However, detailed assessments based on different bio-geographical regions, national parks and sanctuaries, forest types and landscapes were mainly undertaken by Singh (1999), Singh and Bhandari (2003), Joshi *et al.* (1999), and Uniyal (2004). Various studies on insects and status of butterflies of Great Himalayan National Park, Himachal Pradesh were mainly conducted by Uniyal and Mehra (1996), Uniyal and Nagesh Kumar (1997), Uniyal and Mathur (1998), and Uniyal (1996, 1999).

The present study is the first attempt to document the butterfly diversity at the landscape level in the Great Himalayan Conservation Landscape (GHCL) in the districts Kullu and Kinnaur of Himachal Pradesh. The GHCL constitutes areas of the mountainous landscape covering the Great Himalayan National Park, Kanawar, Tirthan, and Rupi Bhaba Wildlife Sanctuary including managed forests of the Parbati Forest Division, Kullu. The study was conducted from March 2002 to July 2003.

Study area – The Great Himalayan Conservation Landscape

The GHCL represents the 2A-North West Himalayas Biotic Province of the 2-Himalayan Biogeographic Zone (Rodgers and Panwar, 1988). The area of GHCL lies in the districts of Kullu and Kinnaur of Himachal Pradesh. The area lies between Latitude 31° 32′ and 32° 14′ 30″ N and Longitude 77° 1′ 30″ to 78° 6′ 30″ E covering 4,854.89 sq km. The constituent areas of the mountainous landscape are the Great Himalayan National Park (754.4 sq km), Pin valley National Park (675 sq km); four Wildlife Sanctuaries *viz.*, Kanawar (63 sq km), Sainj (90 sq km), Tirthan (61 sq km), and Rupi Bhaba (738 sq km); and managed forests of the Parbati Forest Division (2,047 sq km); Ecozone of GHNP (265.49 sq km); and parts of Rampur and Kinnaur Divisions (161 sq km). Thus, GHCL represents one of the largest contiguous tracts under the wildlife protected areas along with adjacent managed forests in the state of Himachal Pradesh (Wildlife Institute of India, 2005).

The landscape features

The terrain in the landscape is characterized by numerous high ridges (>4,000 m), snow capped peaks, large glaciers, deep gorges and precipitous cliffs, and narrow valleys. The GHCL constitutes significant and valuable catchments of two regionally important major rivers *viz.*, Beas and Satluj in the state and its important tributaries are the Parbati, Jiva, Sainj, and Tirthan that drain the landscape. The northern and northeastern parts of the landscape cover several prominent glaciers while the rest of the area is criss-crossed with streams.

An unnamed highest peak is located in the Parbati sub-watershed while the minimum altitude is closer to southern boundary of the landscape i.e. river Satluj. This vast altitudinal gradient along with multiplicity of different landforms, slopes, aspects and past management has provided diversity of forests and other wildlife habitats. Bulk of the temperate forests occurs in lower altitudes (1,300–3,200 m). A narrow belt of sub-alpine forests occurs at >3,200–3,600 m elevation. Alpine pastures at >3,600 m dots the landscape. The landscape is highly significant from biodiversity point of view with a high level of rare and endangered floral and faunal species.

Floral diversity

The flora of GHCL exhibits characteristics of temperate – alpine type (Rawat, 2003). However, the low-lying river valleys and grassy slopes are characterized by sub-tropical elements such as *Toona ciliata, Dalbergia sissoo, Carissa carandas, Woodfordia fruticosa*, and *Ficus* spp. Coniferous trees such as *Pinus roxburghii, Pinus wallichiana, Cedrus deodara, Taxus wallichiana, Picea smithiana, Abies pindrow* and *Abies spectabilis* characterize the temperate belt. Oaks (*Quercus* spp.) form important floral elements in the temperate broadleaf forests. In the sub-alpine zone, *Prunus cornuta, Betula utilis* and *Rhododendron campanulatum* are the important floral elements. The temperate and sub-alpine regions of GHCL also exhibit high diversity of shrub species. Common genera of shrubs in the region are *Berberis*,

Daphne, *Desmodium*, *Deutzia*, *Hypericum*, *Lonicera*, *Indigofera*, *Prinsepia*, *Ribes*, *Rhamnus*, *Rhododendron*, *Rubus*, *Sarcoccoca*, *Sorbaria* and *Viburnum*. Two species of hill bamboo *viz*., *Arundinaria falcata* and *Thamnocalamus spathiflorus* were also found in the study area.

METHODS

The survey was conducted using Pollard walk on fixed transects (Pollard and Yates, 1993) to enumerate the butterfly species in different habitats of GHCL. Existing patrolling paths were used as transects with a minimum of 1 km distance. All flying butterflies on these selected transects were recorded between 0800 to 1000 h. A reference collection was maintained and butterflies that could not be identified were collected and identified later following Evans (1932), Talbot (1939), Wynter-Blyth (1957), Mani (1986) and reference collection at Zoological Survey of India. To control sample size effects, Shannon index was used to calculate species diversity, to emphasize the richness component of butterfly diversity. Species presence/absence data in five different habitat types were analyzed using cluster analysis (Sorensen distance) to reveal similarities between habitat types.

RESULTS AND DISCUSSION

A total of 75 species of butterflies belonging to 48 genera were documented from different altitude and watershed of GHCL (Table 1). Ten species belonging to five genera of family Papilionidae were recorded in different vegetation and forest community. The Common blue apollo (*Parnassius hardwickei*) and Regal apollo (*Parnassius charltonius*) were recorded from the alpine areas above 3,500 m altitude. Fourteen species belonging to ten genera of family Pieridae were recorded from broad leaved forest areas between 1,000 to 2,500 m altitude. Only four species *viz*. Dark clouded yellow (*Colias electo fieldii*); Pale clouded yellow (*Colias erate*); Himalayan blackvein (*Aporia leucodyce*) and Lesser brimstone (*Gonepteryx aspasia*) were found in sub alpine to alpine areas. Family Nymphalidae with 37 species of 23 genera had the largest representation. Most of the species of Nymphalidae were documented from broad leaved forest areas in the landscape. The Indian red admiral (*Vanessa indica*), Painted lady (*Vanessa cardui*), Eastern comma (*Vanessa egea*), Indian tortoise shell (*Aglais cashmiriensis*), Queen of Spain fritillary (*Issoria lathonia*), Large silver strip (*Argynnis childreni*), Comma (*Polygonia c-album*), Great satyr (*Aulocera padma*), Common satyr (*Aulocera swaha*), etc. were the species observed in broad leaved and sub-alpine and alpine area. Ten species belonging to seven genera of family Lycaenidae were documented in broad leaved to mixed broad leaved areas. Four species belonging to three genera of family Hesperiidae were documented in mixed broad leaved forest areas.

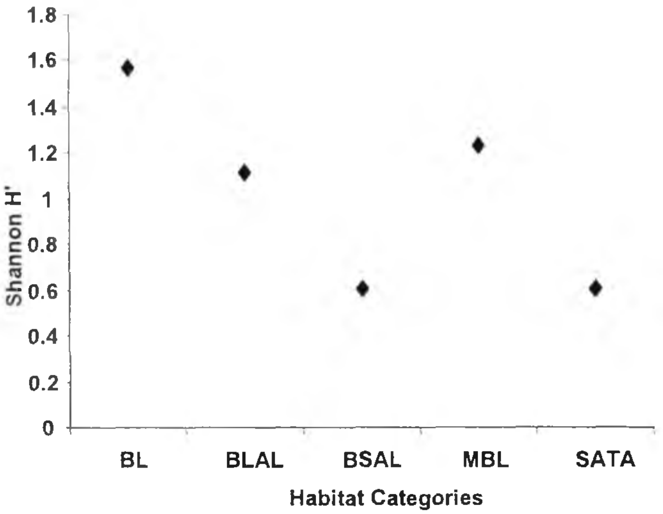

FIGURE 1. Diversity index of butterfly assemblage for different habitats along elevation zones. BL, broad leaved; BLAL, broad leaved to alpine; BSAL, broad leaved to subalpine; MBL, mixed broad leaved; SATA, subalpine to alpine.

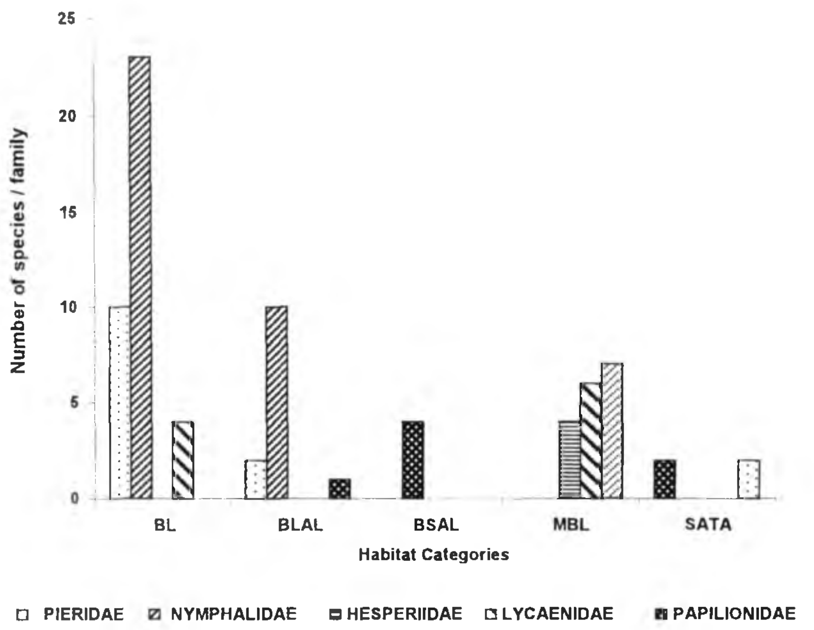

FIGURE 2. Family composition of butterfly assemblage in different habitat categories

TABLE 1. Butterfly species recorded from Great Himalayan Conservation Landscape

Family/species	Common name	Habitat	Altitude (m)
Papilionidae			
Atrophaneura polyeuctes Doubleday	Common Windmill	MBL	1000–2500
Graphium cloanthus Westwood	Glassy Blue Bottle	MBL	1000–2500
Papilio machaon L.	Yellow swallowtail	BLAL	2000–3500
Parnassius charltonisus Gray	Regal Apollo	SATA	3000 & above
P. hardwickei Gray	Common Blue Apollo	SATA	3000 & above
Princeps polyctor Boisduval	Common Peacock	MBL	1000–2500
P. arcturus Westwood	Blue Peacock	MBL	1000–2500
P. demoleus L.	Lime Butterfly	MBL	1000–2500
P. krishna Moore	Krishna Peacock	MBL	1000–2500
P. polytes L.	Common Mormon	MBL	1000–2500
Pieridae			
Anapheis aurota aurota Fabricius	Pioneer	BL	1000–2000
Aporia leucodyce Eversmann	Himalayan Blackvein	BLAL	2000–3500
Catopsilia pomona Fabricius	Lemon Emigrant	BL	1000–2500
Colias electo fieldii Menetries	Dark Clouded Yellow	SATA	2000 & above
C. erate Esper	Pale Clouded Yellow	SATA	2000 & above
Delias belladonna Fabricius	Hill Jezebel	BL	1000–2500
Gonepteryx aspasia Menetries	Lesser Brimstone	BLAL	1000–3500
G. rhamni L.	Common Brimstone	BL	1000–2500
*Parenonia valeria hippie*Fabricius	Common Wanderer	BL	1000–2000
Pieris brassicae L.	Large Cabbage White	BL	1000–2000
P. canidia indica Evans	Indian Cabbage White	BL	1000–2000
P. dubernardi chumbiensis De Niceville	Chumbi White	BL	1000–2000
Pontia daplidice L.	Bath White	BL	1000–2000
Prioneris thestylis thestylis Doubleday	Spotted Sawtooth	BL	1000–2000
Nymphalidae			
Abisara echerius Stoll	Plum Judy	BL	1000–2500
A. fylla Doubleday	Dark Judy	BL	1000–2500
Acraea violae Horsfield	Tawny Coster	BL	1500–2500
Aglais cashmiriensis Kollar	Indian Tortoiseshell	BLAL	1000 & above
Argynnis childreni Gray	Large Silver Stripe	BLAL	2000–3500
A. hyperbius Johanssen	Indian Fritillary	BL	1000–2500
Aulocera padma Kollar	Great Satyr	BSAL	1000–3000
A. saraswati Kollar	Striated Satyr	BSAL	1000–3000
A. swaha Kollar	Common Satyr	BSAL	1000–3000
Cynthia erota Fabricius	Cruiser	BL	1000–2500
Danaus aglea Cramer	Glassy Tiger	BL	1500–2500
D. chrysippus L.	Plain Tiger	BL	1000–2500
D. genutia Cramer	Common Tiger	BL	1000–2500
Dodona durga Kollar	Common Punch	BL	1000–2500
Issoria lathonia issaea Doubleday	Queen of Spain Fritillary	BLAL	2000 & above
Lassiommata schakra Kollar	Common Wall	BSAL	1000–2500
Lethe nicetas Hewitson	Yellow Woodbrown	BLAL	1000–3500

contd...

TABLE 1. (*contd...*)

Family/species	Common name	Habitat	Altitude (m)
Nymphalidae			
L. pulaha Moore	Veined Labyrinth	BLAL	1500–3500
L. verma Fruhstorfer	Straight-Banded Tree Brown	BL	1000–2500
Mycalesis francisca Cramer	Lilacine Bush brown	BL	1500–2500
Neptis hylas varmona Moore	Common Sailer	BL	1000–2500
Parantica sita sita Kollar	Chestnut Tiger	BL	1000–2500
Parathyma perius L.	Common sergeant	BL	1000–2500
Pareba vesta Fabricius	Yellow Coster	BL	1500–2500
Polygonia c-album L.	Comma	BLAL	2000–3500
Precis hierta lemonias L.	Lemon Pansy	BL	1000–2500
P. hierta magna Fabricius	Yellow Pansy	BL	1000–2500
P iphita iphita Cramer	Chocolate Pansy	BL	1000–2500
P. orithyia L.	Blue Pansy	BL	1000–2500
Raphicera moorei Butler	Small Tawny Wall	BLAL	1000–3000
Sephisa dichroa Kollar	Western Courtier	BL	1000–2500
Symbrenthia hypselis Godart	Himalayan Jester	BL	1000–2500
Vanessa canace Johanssen	Blue Admiral	BL	2000–2500
V. cardui L.	Painted Lady	BLAL	2000 & above
V. egea Cramer	The Eastern Comma	BLAL	2000 & above
V. indica indica Herbst	Indian Red Admiral	BLAL	2000 & above
Ypthima baldus Fabricius	Common Five ring	BL	1000–2500
Lycaenidae			
Acetolepsis puspa gisca Fruhstorfer	Common Hedge Blue	MBL	1000–2500
Deudoryx epijarbas Moore	Cornelian	MBL	1000–2500
Heliophorus androcles Hewitson	Green Sapphire	BL	1000–2500
H. bakeri Evans	Western Blue Sapphire	MBL	1000–2500
H. sena Evans	Sorrel Sapphire	MBL	1000–2500
Lampides boeticus L.	Common Pea blue	MBL	1000–2500
Loxura atymnus Cramer	Yam fly	BL	1000–2500
Lycaena phleas L.	Common Copper	BL	1000–2000
Zizeeria lysimon Hubner	Dark Grass Blue	BL	1000–2500
Z. maha Kollar	Pale Grass Blue	MBL	1000–2500
Hesperiidae			
Celaenorrhinus leucocera Kollar	Common Spotted Flat	MBL	1000–2500
Pelopidas sinensis Moore	Large Branded Swift	MBL	1000–2500
Tagiades litigiosa Möschler	Water Snow Flat	MBL	1000–2500
T. menaka Moore	Spotted Snow Flat	MBL	1000–2500

BL, Broad leaved; BLAL, Broad leaved to alpine; MBL, Mixed broad leaved; BSAL, Broad leaved to sub alpine; SATA, Sub alpine to alpine

Habitat heterogeneity and butterfly assemblage

Of the 75 species documented during the survey, 49.3% of species were encountered in broad leaved habitat, which is significantly higher compared to other habitat categories

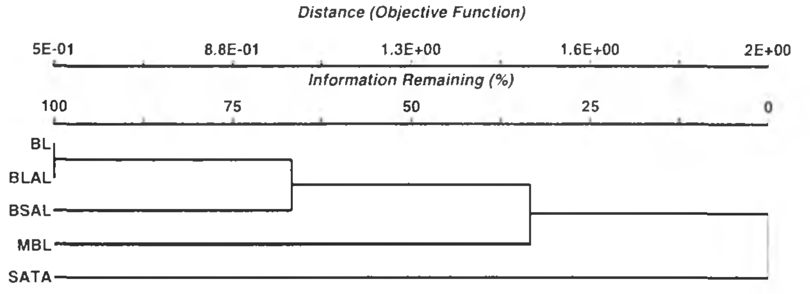

FIGURE 3. Clusters of different butterfly assemblages along elevational gradient based on similarity in butterfly species composition at regional level

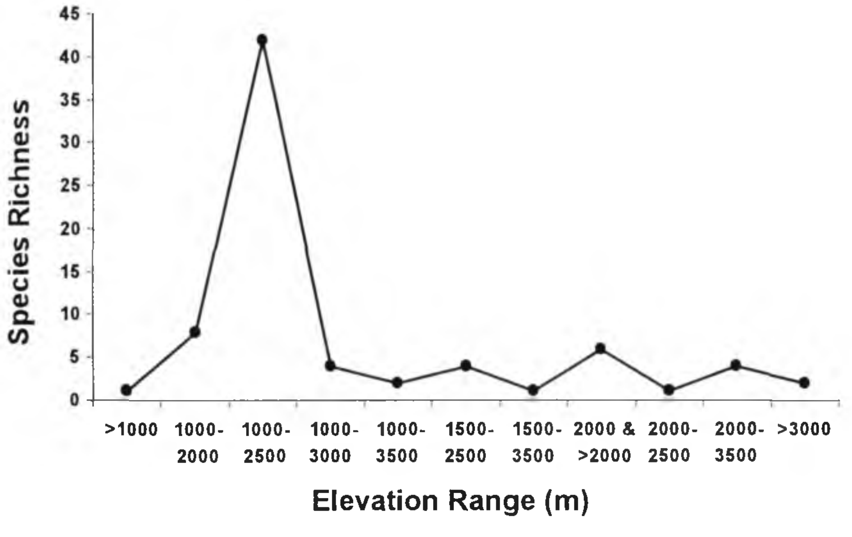

FIGURE 4. Species Richness of butterfly assemblages along 11 elevation zones

viz. broad leaved to alpine, mixed broad leaved, broad leaved to sub alpine and sub alpine to alpine. Shannon index ranked broad leaved habitat as the most diverse and broad leaved to sub alpine as least diverse for butterfly assemblage (Fig. 1). Family Nymphalidae represented highest number of species (37) followed by Pieridae (14), Lycaenidae (10) and Papilionidae (10) (Fig. 2). The cluster analysis of the butterfly assemblage for each habitat (Fig. 3) showed that sub alpine to alpine habitat has the most dissimilar butterfly species followed by mixed broad leaved habitat. The other two main clusters are broad leaved to sub alpine and broad leaved–broad leaved to alpine.

Altitudinal gradient and butterfly assemblage

The empirical species richness did not exhibit a mid-elevation peak for alpha diversity. There was a unimodal pattern, with the peak between 1000–2500 m (Fig. 4). The first peak with respect to other shallower peaks depicts the overall linear increase in species richness with elevation. The elevation zone 1000–2500 m, was found richest in butterfly species representing 56% of total species. Based on species presence/absence data in 11 different elevation zones, cluster analysis (Sorensen distance) was performed to reveal similarities between elevation zones. Cluster analysis identified three broad butterfly assemblages one at 3000 m and above, second at 2500–3000 m and last one grouped all of the remaining nine elevation zones. Elevation zones adjacent to each other had similar species pool and hence the compositions.

ACKNOWLEDGEMENTS

Mr. P. R. Sinha, Director, Dr. V. B. Mathur, Dean and Prof P. K. Mathur, Head, Dept of Landscape Level Planning and Management, Wildlife Institute of India, Dehradun and Himachal Pradesh Forest Department are gratefully acknowledged for necessary support to carry out this study. Thanks to Mr. Upamanyu Hore, Vinay Bhargav and one anonymous reviewer for valuable comments on the earlier draft of the manuscript. The project was funded by Wildlife Institute of India (WII) under Himachal Pradesh Forest Department (HPFD) conservation project of GHCL.

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(Received 8 May 2006; accepted 15 May 2007)

ENTOMON **32(2)**: 129–132 (2007)
Short Communication No. ent.32208

Lac host plants recorded from southern Rajasthan and their relative performance

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ABSTRACT: In a survey conducted in the southern part of Rajasthan, thirteen host plants of lac insect were recorded among which *ber* and *palas* trees were dominant in numbers. Seven host plants *viz*., *Butea monosperma* (Palas), *Zizyphus mauritiana* (Ber), *Ficus religiosa* (Pipal), *Ficus bengalensis* (Bargad), *Cajanus cajan* (Arhar), *Flemingia semialata* and *Flemingia macrophylla* (Bhalia) were evaluated with reference to the quantity of lac produced and developmental parameters. Ber was found to be the best host for lac production as maximum quantity was recorded on it (165.50 g/m.) and also the highest fecundity (525.2 and 450.6 per female), female cell diameter (3.52 and 3.06 mm) and cell weight (14.21 and 10.12 mg) were recorded in *Baisakhi* (summer season) and *Katki* (rainy season) crop, respectively. *Eublemma amabilis* (Moore) (Lepidoptera: Noctuidae) and *Pseudohypatopa pulverea* Meyr. (Lepidoptera: Blastobasidae) were recorded as the major pests of lac insect.

KEYWORDS: Indian lac insect, *Kerria lacca*

Lac is a natural resin produced mainly by Indian lac insect *Kerria lacca* (Kerr.), a soft bodied insect belonging to coccid group of order Homoptera. Lac is mainly produced in India, Thailand, Indonesia, and China. In India, most of the lac cultivation is done by the tribals of Jharkhand, Chhattisgarh, West Bengal, Maharashtra, Madhya Pradesh, Orissa, Gujarat and Assam. On an average, India produces 18 thousand tons of lac per year (Prasad *et al*., 2004). *Rangeeni* and *Kusmi* are two strains of this insect, each of these produce two crops in a year (bi-voltine). *Kusmi* strain grows well on *Kusum* tree (*Schleichera oleosa*) and also on other trees but not on *Palas* (*Butea monosperma*) whereas Rangeeni strain grows well mainly on palas and also on a few other trees but not on *Kusum* tree.

To find out the different host plants of lac insect, a survey was conducted in Udaipur, Dungarpur, Chittorgarh and Rajsamand districts of southern Rajasthan. To evaluate

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TABLE 1. Yield of scraped *rangeeni* lac on different host plants

Host Plant	Yield (dry) g/m. length	
	Baisakhi	*Katki*
Palas, *Butea monosperma*	150.75	91.42
Ber, *Zizyphus mauritiana*	160.50	102.50
Bargad, *Ficus bengalensis*	145.00	93.64
Pipal, *Ficus religiosa*	152.25	94.80
Arhar, *Cajanus cajan*	134.83	82.26
Flemingia semialata	141.97	78.02
Bhalia, *Flemingia macrophylla*	140.80	75.25

the relative preference of *K. lacca,* seven different host plants were selected and evaluated on the basis of quantum of lac produced on the stick of one meter length and one centimeter diameter of randomly selected five branches of the host. On these selected hosts the data obtained for the developmental parameters like average life span, fecundity (average of 25 cells), female cell diameter (average of 100 cells) and female cell weight (average of 100 cells) for two crops i.e. *Baisakhi* (summer season) and *Katki* (rainy season) were collected. In the survey, key biotic mortality factors were also recorded and identified with their management practices without affecting the quality of lac.

In the survey, thirteen different host plants *viz*., palas (*Butea monosperma* Lam.), ber (*Zizyphus mauritiana* Lam. and *Z. jujube* Lam.), pipal (*Ficus religiosa* Linn.), Bargad (*Ficus bengalensis* Linn.), paras pipal (*Ficus benjamina* Linn.), calandra (*Calandra* sp.), siris (*Albizzia lebbek* Benth), custard apple (*Annona squamosa* Linn.), khair (*Acacia catechu* Willd.), arhar (*Cajanus cajan* Linn.), gular (*Ficus racemosa* Linn.), babool (*Acacia arabica* Willd.) and amaltas (*Cassia fistula* Linn.) were recorded on the basis of natural lac culture. Ber trees followed by palas trees were recorded in maximum numbers (102) in different regions, whereas, Palas (28) pipal (26), bargad (33), paras papal (11), custard apple (16) were found in moderate numbers. The findings are in conformity with Singh and Chatterjee (1994) who reported *Z. mauritiana* and *B. monosperma* as the major lac hosts. Similarly Kumar and Chauhan (1976) reported *Cajanus cajan* as the possible host of lac insect and Jaiswal *et al*., 2003 reported that in lac growing states of Jharkhand, West Bengal and Orissa the maximum number of house hold (84%) utilized ber trees followed by palas (72%) and kusum (57%) for lac production.

Performance of seven host plants *viz*., Palas, Ber, Pipal, Bargad, Arhar, *F. semialata* and *F. macrophylla* was assessed on the basis of quantity of lac produced and also on the basis of developmental parameters of these hosts. *F. semialata* and *F. macrophylla* were introduced in Rajasthan from Indian Lac Research Institute, Namkum, Ranchi. Maximum quantity was recorded on ber (165.50 g/m.) followed by pipal (152.25 g/m.) whereas minimum quantity was recorded from arhar (134.83 g/m) *baisakhi* crop. Hence, on the basis of yield parameters (Table 1) ber was judged as the best host for

TABLE 2. Relative performance of lac hosts on the basis of lac insect developmental parameters

Host	Crop	Life (days)	Fecundity (number)	Female cell diameter (mm)	Live cell weight (mg)
Palas	*Baisakhi*	242.6	506.2	3.45	14.06
	Katki	120.4	402.0	2.90	9.95
Ber	*Baisakhi*	234.2	525.2	3.52	14.21
	Katki	119.8	450.6	3.06	10.12
Bargad	*Baisakhi*	246.2	473.4	3.43	13.95
	Katki	118.1	380.0	2.88	9.70
Pipal	*Baisakhi*	240.2	503.8	3.48	14.18
	Katki	121.3	415.2	2.95	10.09
Arhar	*Baisakhi*	247.1	409.0	3.27	13.60
	Katki	116.2	315.4	2.60	9.40
F. semialata	*Baisakhi*	248.4	467.6	3.38	13.70
	Katki	120.2	345.2	2.86	9.51
Bhalia,	*Baisakhi*	246.2	460.8	3.35	13.67
F. macrophylla	*Katki*	123.6	338.0	2.85	9.49

lac production. On the basis of developmental parameters, (Table 2) minimum life span of 234.2 and 119.8 days were recorded on *Baisakhi* and *Katki* crops respectively on ber while maximum fecundity (525.2 and 450.6 per female), female cell diameter (3.52 and 3.06 mm) and live cell weight (14.21 and 10.12 mg) were recorded on ber in *Baisakhi* and *Katki* crop respectively. Therefore, ber can be confirmed as the best suitable host for lac cultivation in southern Rajasthan. These findings were found in agreement with Singh and Chatterjee (1994).

The natural enemies or biotic factors which were recorded on lac insects and were identified from Indian Lac Research Institute, Namkum, Ranchi, are: *Eublemma amabilis* (Moore) (Lepidoptera: Noctuidae), *Pseudohypatopa pulverea* Meyr. (Lepidoptera: Blastobasidae), *Chrysopa* spp. (Neuroptera: Chrysopidae) *Ephestia* sp. (Lepidoptera: Pyralidae), *Tachardiaephagus tachardiae* (How.) (Hymenoptera: Encyrtidae), *Euplemus tachardiae* (How.) (Hymenoptera: Eupelmidae), *Aprostocetus (Tetrastichus) purpureus* (Cam.) (Hymenoptera: Eulophidae) and *Apanteles tachardiae* (Hymenoptera: Braconidae). The present studies are in conformity with Sushil *et al.* (2002) and Sharma and Jaiswal (2002).

ACKNOWLEDGEMENTS

The authors are thankful to the scientists at Indian Lac Research Institute, Ranchi and Dean, Rajasthan College of Agriculture, Udaipur for providing the necessary facilities.

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(Received 9 February 2007; accepted 15 May 2007)

ENTOMON **32(2)**: 133–135 (2007)
Short Communication No. ent.32209

Biology and morphometrics of *Dipha aphidivora* Meyrick (Lepidoptera: Pyralidae), a potential predator of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner

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ABSTRACT: The aphid predator, *Dipha aphidivora* (Lepidoptera: Pyralidae) has five larval instars. The larval durations for first, second, third, fourth and fifth instars are 3.3, 3.3, 3.0, 2.4 and 2.3 days respectively. Pupation occurs in white cocoons. Female lays 59 to 96 oval, creamy white eggs singly on the underside of leaf. The life span of female is 30 days, and of male 27 days.

KEYWORDS: biology, morphometrics, sugarcane woolly aphid, *Dipha aphidivora*

The sugarcane woolly aphid (SWA), *Ceratovacuna lanigera* Zehntner has attained pest status in the year 2004 and appeared in epidemic form in southern states of India. Later it spread to Tamil Nadu becoming a nightmare for cane farmers. Many predators were spotted along with SWA. Among them, *Dipha aphidivora* Meyrick (Lepidoptera: Pyralidae) is the most potential and very little of its life history is known. In this context, the present investigation was undertaken.

The biology and morphometrics of *D. aphidivora* were studied under laboratory conditions at the mean temperature and relative humidity of 27 °C and 83 per cent respectively, during July 2005. The adults of the predator were obtained from mass rearing of *D. aphidivora* in the laboratory. Ten pairs each of adults were released in plastic containers and were provided with 10% sugar solution enriched with ABDEC vitamin drops. The plastic container was covered with black muslin cloth which also served as oviposition substrate. Muslin cloths with eggs were kept in plastic containers and the neonate larvae were kept individually on small leaf bits which were inserted in the polypots containing agar medium to maintain turgidity of the leaf. Whenever the

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TABLE 1. Biometric data of *D. aphidivora*

Life stage	Duration (days)	Body length (mm)	Body width (mm)	Head capsule width (mm)
Egg	3.27 ± 0.92	1.10 ± 0.32	1.09 ± 0.31	–
First instar	3.30 ± 1.19	1.42 ± 0.27	1.33 ± 0.19	0.941 ± 0.017
Second instar	3.30 ± 0.15	3.15 ± 1.00	1.75 ± 0.31	1.299 ± 0.036
Third instar	3.00 ± 1.53	5.70 ± 0.36	2.68 ± 0.38	1.761 ± 0.034
Fourth instar	2.40 ± 1.23	9.99 ± 0.96	3.36 ± 0.50	2.357 ± 0.060
Fifth instar	2.30 ± 0.92	10.69 ± 0.92	3.81 ± 0.25	2.922 ± 0.024
Prepupa	1.33 ± 0.92	8.57 ± 0.31	4.37 ± 0.08	–
Pupa	5.60 ± 1.03	6.25 ± 0.59	2.70 ± 0.44	–
Adult male	3.33 ± 1.03	7.52 ± 0.31	–	–
Adult female	5.87 ± 1.55	11.99 ± 0.19	–	–

Mean of 30 samples

leaf bit turned yellow, it was replaced with fresh leaf bits. About 300 polypots with leaf bits and larvae were maintained.

The head capsule width of larvae were measured daily by image analyzer by destructive sampling and larval instars were determined by Dyar's law. The pupae were kept separately and on moth emergence, pupal period was recorded. After the adult emergence, adult longevity, fecundity, pre-oviposition, oviposition and egg periods were noted. The morphometric data for egg, larval instars and pupa were recorded by using ocular micrometer.

The eggs were laid singly. Freshly laid eggs are small, oval and creamy white. Two days before hatching, they changed to pale yellow and one day before hatching, a spot developed at the micropyle region of the egg. The egg hatchability is 95.57 per cent.

The newly hatched larva is yellowish white and the head capsule is light brown. Hairs are present on the body and are visible only under microscope. The second instar larva is similar to the first instar. The differentiation between head and thoracic regions is not discernible even under microscope, but can be done through image analyzer. In the third instar the colour of the head capsule intensified to dark brown. Hairs are visible to the naked eye. The larva is light green. The fourth instar larva resembled the third instar in appearance, but the abdomen ended bluntly. The fifth instar larva is similar to the fourth instar but for the size. The total larval period lasts 14.3 days. At the prepupal stage, the larva stops feeding and become sluggish and the body shrunken. The pupa is cylindrical and reddish brown. It forms a loose white cocoon around it. Larval mortality was very low with 98.9 per cent pupating.

The adult is ash brown in colour. The forewings have two black spots one on each, on the posterior region of the wing. Hind wings are transparent and grayish white. In female, the fore and hind wing expanses are 7.45 mm and 6.09 mm respectively. In male, the fore and hind wing expanses are 6.05 and 4.32 mm respectively. Males are

usually smaller. Adult emergence occurred between 19.30 and 20.30 hrs. Nearly 96 per cent of adults emerged.

The premating period ranged from 1.25 to 2.40 h with an average of 1.86 h. Mating occurred mostly during night. Preoviposition period ranged from 2 to 3 days with a mean of 2.67 days. The oviposition period lasted for 2 to 3 days with an average of 2.33 days. The total number of eggs laid by a female moth ranged from 59 to 96 with an average of 91.8. The mean male longevity is 3.33 days and mean female longevity 5.87 days. The total duration of life cycle of male ranged from 25 to 27 days with a mean of 25.53 days and of female ranged from 28 to 30 days with a mean of 28.93 days.

By comparing the body measurements and the head capsule widths (Table 1), it was found that *D. aphidivora* underwent five larval instars. The larval duration for first, second, third, fourth and fifth instars were 3.3, 3.3, 3.0, 2.4 and 2.3 days respectively. In contrast, *D. aphidivora* had only four larval instars in Japan (Arakaki and Yoshiyasu, 1988). Five larval instars were also reported from Karnataka (PDBC, 2006).

ACKNOWLEDGEMENTS

The financial support by PDBC is gratefully acknowledged.

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(Received 3 January 2007; accepted 15 May 2007)

ENTOMON **32(2)**: 137–141 (2007)
Short Communication No. ent.32210

Developmental biology of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee in mid-hills of Himachal Pradesh

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ABSTRACT: In the mid-hill zone of Himachal Pradesh, *Leucinodes orbonalis* completed 8–9 overlapping generations per year. The larval period was 12–18 days in most of the generations except the winter generation in which the fifth instar caterpillars overwintered for a period of about 134 days. The total life cycle was completed in 22–30 days between March and September but there was large variation between November and February. The duration of each of the developmental stage for each generation has been worked out. © 2007 Association for Advancement of Entomology

The brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee is the most destructive pest of brinjal throughout India. The pest has been reported to inflict losses to the tune of 20.7–80.0 per cent from different parts of the country (Lal *et al.*, 1976; Raja *et al.*, 1999; Sasikala *et al.*, 1999; Jhala *et al.*, 2003). The biology of the pest has been studied in different parts of the country (Allam *et al.*, 1982; Singh and Singh, 2001a; Jat *et al.*, 2003). However, no detailed and systematic work has been carried out in Himachal Pradesh. Therefore, the studies were undertaken on the detailed developmental biology of this pest in the mid-hill tracts of Himachal Pradesh.

Biology of *L. orbonalis* was studied under laboratory conditions on fruits of brinjal variety, Arka Nidhi during 2003–04 at Palampur (1290 m amsl), representing mid-hill zone of Himachal Pradesh. The laboratory culture was initiated from the field-collected caterpillars. The adults were fed with 10 per cent honey solution on a cotton swab. The freshly hatched larvae were transferred to brinjal fruits and the fruits were changed at periodic intervals to avoid growth of saprophytes. Observations on the fecundity of female moths were recorded and hatchability and incubation period were worked out. The observations on the developmental biology were recorded throughout the year by observing a cohort of 100 larvae. The duration of larvae, pupae and total developmental period along with survival in respective developmental stage and longevity of male and female moths were recorded. Observations on the survival of first and second instar larvae were taken at the end of second instar owing to the small

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size and internal feeding habit of the first instar. Temperature and relative humidity prevailing during the course of study were recorded using thermo-hygrograph.

L. orbonalis completed 8–9 overlapping generations in a year in the mid-hill conditions of Himachal Pradesh (Table 1). Number of generations produced in a year is mainly dependent upon the agro-climatic conditions prevailing in a particular region. The present findings are in conformity with the observations of Taley *et al.* (1984), who found 8–9 generations of *L. orbonalis* in Maharashtara. Lall and Ahmad (1965) and Singh and Singh (2001c) observed 10 and 8 generations in Bihar and Meghalaya respectively.

The incubation period varied between 2–8 days in different generations, with the mean duration ranging between 3.0–6.8 days. This variation could be attributed to prevalence of low temperature in the winter generation (the maximum and minimum temperature being 14.7 and 12.8 °C, respectively), signifying the impact of low temperature in prolonging incubation period. Taley *et al.* (1984), Baang and Corey (1991), and Suresh *et al.* (1996) also reported the incubation period of *L. orbonalis* to vary between 3 to 7 days. Egg hatchability varied between 34.8–85.3 per cent, maximum being in the generation occurring during August (G_V), which was at par to August–September (G_{VI}) and July–August (G_{IV}), which more or less coincide with the earlier findings of Baang and Corey (1991), Yin (1993) and Singh and Singh (2001b) who found egg hatchability to range between 57.5–82.6 per cent.

There were five larval instars, the duration of fifth instar being longest (4.0–106.0 days) compared to the durations of first four instars being 1.8–4.8, 2.2–6.0, 2.6–8.0 and 2.2–9.0 days respectively. The larval duration was significantly higher in November to March (G_{VIII}) followed by September to March (G_{VII}). The duration of larval instars finds partial support from the findings of Singh and Singh (2001b).

The total larval period was completed in 12–18 days in most of the generations except the winter generation (G_{VIII}) where fifth instar caterpillars underwent over-wintering and resulted in prolonged duration of 133–135 days which falls within the range of larval duration as reported by Mehto *et al.* (1983), Taley *et al.* (1984) and Singh and Singh (2001b). Full-fed larvae overwintered from November to March outside the fruit in brown coloured pupal case and transformed into pupae in the last week of March and subsequently adult emergence took place. Panwar (1995) and Atwal and Dhaliwal (2002) also reported the *L. orbonalis* caterpillars to hibernate in winter and pupate early in spring. But, in China and Meghalaya, *L. orbonalis* was observed to overwinter in pupal stage (Yin, 1993; Singh and Singh, 2001c).

The observations on the survival among different larval instars revealed the first two experiencing lowest survival as compared to other instars in all the generations varying between 45.0 to 77.0 per cent as compared to others (87.3–100%) being maximum in fifth instar. The total larval survival was maximum (71.0%) during June–July (G_{III}) being on par to that observed in May–June (G_{II}) and September–October (G_{VII}) generations. It was found to be minimum (32.0%) during the generation occurring from November to March (G_{VIII}), being at par to that in April–May (G_I).

TABLE 1. Developmental biology of *Leucinodes orbonalis* during different generations

Developmental stage		Generation									CD
		I	II	III	IV	V	VI	VII	VIII	IX	(0.05)
Egg	Duration*	5.0(5-6)	3.2(3-5)	4.0(3-5)	3.2(3-5)	3.0(2-4)	4.0(3-5)	4.2(4-6)	6.8(6-8)	6.0(4-6)	0.49
	Hatchability**	34.8(34.4)	39.0(38.1)	43.0(40.7)	72.3(60.2)	85.3(68.0)	77.2(61.7)	59.3(50.9)	56.9(49.4)	37.6(37.3)	(16.7)
Larval instar											
I	Duration	2.2(2-3)	2.0(2-3)	1.8(1-3)	2.0(2-3)	2.0(2-3)	2.0(2-3)	2.4(2-3)	4.8(4-6)	2.0(2-3)	0.51
II	Duration	2.6(2-3)	2.2(2-3)	2.2(2-3)	2.2(2-3)	2.4(2-3)	2.4(2-3)	3.2(2-4)	6.0(5-7)	2.4(2-3)	0.74
	Survival(I-II)*	59.0(50.2)	71.0(57.4)	77.0(61.8)	58.0(49.6)	58.0 (49.7)	54.0(47.4)	71.0(57.5)	45.0(42.1)	51.0(45.6)	(6.7)
III	Duration	3.4(3-4)	2.6(2-3)	2.6(2-3)	3.0(2-4)	3.0(2-4)	3.2(3-4)	4.4(4-5)	8.0(7-10)	2.8(2-3)	0.87
	Survival	89.6(75.5)	92.8(78.0)	93.2(76.6)	92.8(78.1)	90.8(74.4)	91.9(75.2)	92.5(76.0)	90.8(74.1)	88.0(74.8)	(NS)
IV	Duration	3.2(3-4)	2.6(2-3)	2.2(2-3)	2.8(2-3)	2.8(2-3)	2.8(2-3)	4.6(4-6)	9.0(8-10)	3.2(3-4)	0.78
	Survival	98.2(86.5)	98.6(86.9)	98.5(86.7)	97.5(85.8)	98.0(86.3)	97.5(85.8)	94.3(81.2)	87.3(69.1)	94.4(81.3)	(NS)
V	Duration	4.6(4-6)	4.4(4-5)	4.2(4-5)	4.0(3-5)	4.6(3-6)	4.6(4-5)	7.0(6-10)	106.0(104-110)	4.2(4-5)	1.52
	Survival	98.5(86.7)	100.0(90.0)	100.0(90.0)	100.0(90.0)	100.0(90.0)	100.0(90.0)	98.5(86.7)	87.8(74.2)	97.8(86.1)	(8.8)
Total larva duration		16.0(15-18)	13.8(13-15)	13.0(13-14)	14.0(12-16)	14.8(13-18)	15.0(13-17)	21.8(18-28)	133.8(133-135)	14.6 (14-17)	2.33
	Survival	51.0(45.5)	65.0(53.8)	71.0(58.0)	53.0(46.7)	52.0(46.2)	49.0(44.4)	62.0(52.2)	32.0(34.2)	45.0(42.1)	(8.5)
Pupa	Duration	7.0 (6-8)	6.2 (5-8)	6.6 (6-8)	6.4 (6-7)	6.8 (6-7)	7.8 (7-8)	7.0 (7-8)	8.8 (7-10)	7.2 (5-8)	1.06
	Survival	93.3(78.3)	88.3(72.1)	98.3(86.6)	92.2(79.5)	97.5(85.8)	94.1(80.9)	72.3(58.8)	55.2(48.3)	83.6(71.2)	(16.3)
Total developmental period*		28.0 (27-29)	23.2 (22-25)	23.6 (23-25)	23.4 (22-25)	23.2 (22-26)	28.4 (26-30)	36.4 (33-37)	149.0 (147-150)	25.2 (21-27)	1.88
Adult emergence**		16.9(23.5)	19.5(25.3)	27.0(30.8)	36.9(37.2)	37.9(37.9)	35.5(36.2)	30.4(32.8)	9.4(17.2)	6.4(14.1)	(9.6)
Longevity*	Male	1.9(1-4)	2.7(1-4)	1.7(1-3)	3.4(2-5)	3.9(1-6)	4.3(3-6)	3.5(2-8)	4.1(2-6)	2.8(1-5)	1.13
	Female	2.8(2-4)	3.9(3-5)	2.9(2-3)	4.4(2-6)	4.3(3-6)	4.8(3-9)	4.1(2-9)	4.7(2-8)	2.8(2-4)	1.32
Fecundity***		134.6	162.5	136.3	186.3	101.3	164.1	133	78.8	32.6	55.71

Generation: G_{I} (April 19–May 17, 03), G_{II} (May 19-June 11), G_{III} (June 13-July), G_{IV} (July 9-Aug. 1), G_{V} (Aug. 3-Aug. 26), G_{VI} (Aug. 28-Sept. 25).

G_{VII} (Sept. 27-Oct. 30) G_{VIII} (Nov. 1–Mar.29, 04), G_{IX} (Mar. 31–April 22, 04).

* (in days) Figures in parentheses are the range values.

** (in %) Figures in parentheses are the angular transformed values.

*** (mean number of eggs laid/female).

The pupal period varied from 6.2–8.8 days in different generations, maximum being in the generation occurring in November–March. Among the different developmental stages, pupal stage experienced the least mortality and resulted in survival varying from 55.2–98.3 per cent in different generations, the minimum and maximum corresponding to generations occurring in November–March (G_{VIII}) and June–July (G_{III}), respectively. Mehto *et al.* (1983) also reported the pupal period of *L. orbonalis* to be 9.8 days. However, Allam *et al.* (1982) and Singh and Singh (2001b) observed the pupal period of 7–11 and 10.4 days in Andhra Pradesh and Meghalaya, respectively.

The life cycle of *L. orbonalis* from egg deposition to adult emergence was completed in 22–30 days in all the generations occurring during March to September. However, there existed a considerable variation in developmental period during November–March generation, which was the longest (149.0 days). The earlier report of Allam *et al.* (1982) and Singh and Singh (2001b) on the duration of life cycle indicated it to vary from 19–28 and 30.7–76.1 days, respectively. This difference can be attributed to the variation in the prevailing climatic conditions. Singh and Singh (2001c) also observed the duration of life cycle of *L. orbonalis* to extend during winters in Meghalaya, but the corresponding duration was quite low (76.1 days) in comparison to the present observations. The total survival during different generations was low to moderate (6.4–37.9%). Significantly low survival was observed in generations occurring during November to May ($G_{VIII,IX,I}$) when temperature was low indicating a pronounced effect of weather factors affecting generation survival.

The females lived longer than males; the longevity varying between 2.8–4.8 and 1.7–4.3 days, respectively, in different generations. However, Allam *et al.* (1982), Singh and Singh (2001b) and Jat *et al.* (2003) recorded the longevity of males and females as 1–2 and 2–3; 3.5 and 5.8; and 1.8 and 3.1 days, respectively. The males outnumbered the females and overall sex ratio of 1: 1.3 (females: males) was observed which is contrary to the findings of Taley *et al.* (1984), who observed sex ratio to be in favour of females (2:1). The mean fecundity of different generations (125.5 eggs/female) under present studies is almost in proximity to that of Baang and Corey (1991), and Nwana (1992) who reported it to be 121.5 and 123–137 eggs, respectively. However, observations of Mehto *et al.* (1983) on the fecundity (84.5–253.5) of *L. orbonalis* are contrary to the present findings.

In the present study, it was observed that the last larval instar experiencing low temperature underwent overwintering, while in the other generations the maximum temperature was $>$ 20 °C, signifying the strong influence of temperature on the biology of the pest. Relative humidity did not play crucial role in influencing overwintering of *L. orbonalis* in the present observations. From the above discussion, it can be conjectured that if temperature is conducive and host plant is available *L. orbonalis* may continue to breed throughout the year.

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(Received 4 January 2007; accepted 15 May 2007)

ENTOMON **32(2):** 143–147 (2007)
Short Communication No. ent.32211

Genotype × environment interaction in the silkworm, *Bombyx mori* L.

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ABSTRACT: Six multivoltine and six bivoltine breeds of the silkworm (*Bombyx mori* L.) drawn from working germplasm at Central Sericultural Research and Training Institute, Mysore along with their F_1 hybrids were evaluated for various quantitative characters during three different seasons of the year. Among multivoltine breeds, BL_{67} and BL_{68} were found good general combiners exhibiting significant general combining ability (GCA) effects for twelve characters. Among bivoltine breeds, CSR_2 was found good combiner expressing significant GCA effects for eleven characters. Genotype × environment (G× E) interaction revealed greater mean square values for lines vs. testers.

KEYWORDS: *Bombyx mori* L., general combining ability effects, genotype × environment interaction

The development and identification of genotypes of *Bombyx mori* L. that have consistent performance in a wide range of environmental conditions are desirable (Hallauver, 1987; Iyengar *et al.*1983). Studies on genotype × environment (G × E) interaction have been carried out in silkworm (Harada *et al.*, 1961; Krishnaswami and Narasimhanna, 1974; Giridhar *et al.*, 1990; Das *et al.*, 1995; Kalpana and Srirama Reddy, 1998; Rao *et al.*, 2004). It is necessary to evaluate a large number of genotypes over a wide range of environmental conditions in order to know their yield potential so as to use them in future breeding programmes. The present study describes the performance of crosses made between multivoltine and bivoltine silkworm breeds/hybrids under different environmental conditions.

Six multivoltine silkworm breeds namely, BL_{67}, BL_{68}, 96A, 96E, 96H and PM and six bivoltine breeds viz., CSR_2, CSR_3, CSR_4, CSR_{12}, CSR_{17} and NB_4D_2 were selected from the working germplasm of Central Sericultural Research and Training Institute, Mysore and were used as lines and testers, respectively. Crosses were made between multivoltine and bivoltine breeds raising thirty six hybrids. F_1 hybrids along with parents were reared thrice with three replications during summer, monsoon and

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TABLE 1. Pooled general combining ability effects of multivoltine and bivoltine silkworm breeds

Lines/ Testers	Fecun- dity	Hatching percentage	Total larval duration	Pupation rate	Yield/10,000 larvae by wt.	Cocoon weight	Cocoon shell weight	Cocoon shell percentage	Filament length	Reel ability	Raw silk percentage	Denier	Neatness
Multivoltine silkworm breeds (Lines)													
BL_{67}	27.63**	0.45**	−1.33**	211.41**	1.31**	0.09**	0.02**	0.39**	47.85**	0.81**	0.32**	0.04	0.91**
BL_{68}	30.69**	0.34**	−7.11**	189.43**	1.05**	0.05**	0.02**	0.44**	19.83**	1.09**	0.54**	0.05	0.54**
96A	8.98**	0.21**	0.67	−166 − 95	−0.17	−0.02	0.01*	0.74**	−3.26	0.11	0.31**	0.01	−0.05
96E	−3.13	−0.04	0.85	−135.85	−0.21	−0.04	0.002	0.45**	−20.19	0.07	0.19**	0.04	−0.05
96H	−32.22	−0.21	1.29	−104.36	−0.84	−0.03	−0.01	−0.45	2.78	−0.80	−0.26	−0.08**	−0.10
PM	−31.95	−0.76	5.62	6.30	−1.15	−0.05	−0.03	−1.57	−47.02	−1.28	−1.10	−0.05**	−1.24
Bivoltine silkworm breeds (Testers)													
CSR_2	14.20**	0.36**	8.12	55.28**	0.46**	0.03**	0.02**	0.38**	33.15**	0.57**	0.46**	0.06	0.62**
CSR_3	9.29**	−0.05	−0.70*	−17.04	−0.28	0.002	−0.01	−0.01	−13.49	−0.47	−0.15	−0.09**	0.19*
CSR_4	2.18	0.09	1.18	−85.36	−0.22	−0.04	−0.01	0.02	−10.41	0.41**	−0.08	−0.09**	−0.49
CSR_{12}	−14.27	0.07	0.23	49.99*	0.07	0.02**	0.003	−0.06	7.92**	−0.54	0.09*	0.04	0.04
CSR_{17}	1.34	−0.38	2.23	44.86	0.16**	0.03**	0.004	−0.10	−4.13	0.48**	−0.09	0.05	−0.23
NB_4D_2	−12.76	−0.09	5.18	−47.74	−0.20	−0.04	−0.02	−0.23	−13.03	−0.46	−0.4	0.03	−0.12
CD at 5%	5.06	0.14	0.65	40.08	0.09	0.007	0.004	0.09	4.47	0.29	0.07	0.010	0.14
CD at 1%	6.66	0.18	0.85	52.79	0.12	0.009	0.01	0.01	5.89	0.38	0.09	0.013	0.19

* and ** denote significant difference at 5% and 1% level respectively.

TABLE 2. Pooled analysis of variance of Genotype × Environment interaction for different characters in the silkworm. *Bombyx mori* L.

Source	df	Fecundity	Hatching percentage	Total larval duration	Pupation rate	Yield/ 10,000 larvae	Cocoon weight	Cocoon shell weight	Cocoon shell percentage	Filament length	Reelability	Raw silk percentage	Denier	Neatness
Replications	3	89.77	0.07	8.18	30092.15	0.06	0.001	0.00	0.12	379.56	2.49	0.06	0.00	1.625**
Environments	2	23112.97**	3.64**	333038.00*	891879.00*	96.29**	0.66**	0.03*	9.35**	102660.60*	1715.170**	0.26	0.02**	10.880**
Replication x Environment	6	279.02	0.712	19.35	91700.36	0.12	0.01	0.00	0.53**	185.02	3.80	0.27**	0.01**	0.255
Treatments	47	11871.51**	7.47**	2762.52**	587864.20*	36.95**	0.46**	0.03***	23.35**	20970.80**	43.24**	20.02**	0.57**	50.293**
Parent	11	14668.53**	10.26**	8261.52**	475954.50*	35.19**	0.64**	0.07**	70.98**	306472.80*	122.57**	66.93**	1.68**	185.881**
Lines	5	5237.855**	6.91**	17144.68**	745485.60*	19.22**	0.13**	0.01**	26.75**	62187.08**	151.59**	29.39**	0.37**	303.014**
Testers	5	17213.97**	9.18*8	995.56**	300418.20*	10.55**	0.05*	0.01**	17.81**	5662.82**	35.30**	9.11**	0.23**	0.889
Lines vs. Testers	1	48914.70**	32.39**	175.56**	5980.44	238.27**	6.19**	0.68**	558.04**	3031952.00	413.78**	543.74**	15.55**	525.174**
Parent *vs.* hybrid	1	382.51	99.48**	16993.96**	5250877.00	865.73**	12.85**	0.35**	3.89**	5794910.00	22.89**	0.79**	1.16**	8.333**
Hybrid	35	11320.71**	3.96***	627.65**	489806.90*	13.83**	0.06**	0.01**	8.94**	19736.14**	18.89**	5.83**	0.20**	8.878**
Line effect	5	55503.32**	14.22***	1247.92*	1984796.00	70.59**	0.21*	0.03*	54.15**	766.70*	59.93**	26.13**	0.22	38.510**
Tester effect	5	9520.15*	4.38	1436.21**	250925.20	5.93	0.07	0.01*	3.01*	23599.18*	20.89	4.54	0.33	10.482**
Line × Tester effect	25	2844.29***	1.82**	341.88**	238585.30*	4.05**	0.02**	0.01*	1.08**	7589.27**	10.28***	2.03**	0.17**	2.631**
Error	423	444.51	1.13	20.04	52524.04	0.14	0.001	0.000	0.17	366.85	1.81	0.09	0.00	0.399

*and **denote significant difference at 5% and 1% level, significantly.

winter seasons of the year. After third moult, three hundred larvae were retained in each replication and reared up to spinning. Young age rearing was carried out at 28 ± 1 °C and $85 \pm 5\%$ relative humidity (RH) while late age rearing was conducted at 25 ± 1 °C and $65 \pm 5\%$ RH. Data were recorded for thirteen characters viz., fecundity, hatching percentage, total larval duration, yield/10,000 larvae, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length, reelability, raw silk percentage, denier and neatness. Kempthorne's line × tester approach (1957) was followed using multivoltine breeds as lines and bivoltine breeds as testers to understand Genotype × Environment interaction to select promising multivoltine and bivoltine breeds.

The pooled general combining ability (GCA) effects of multivoltine and bivoltine silkworm breeds for different characters demonstrated that two breeds, BL_{67} and BL_{68} were significantly good general combiners for all the thirteen characters under study except denier followed by 96A for five characters (Table 1). Among the bivoltines, CSR_2 exhibited significant GCA effects for eleven characters except total larval duration and denier followed by CSR_3 for only four characters.

Analysis of variance resulted for G×E interaction revealed significant interaction for treatments, parents, lines, hybrids and lines × tester effect for all the characters (Table 2). Further partitioning of G×E interaction revealed significant mean square for environment for all the characters except raw silk %, for testers except neatness and for lines vs. testers except pupation rate. However, no significant mean square values were observed for replications in all the characters except neatness. Replication × environment also revealed non-significant values for most of the characters except cocoon shell percentage, raw silk percentage and denier. Maximum significant mean square value was found for lines vs. testers for five characters viz., cocoon shell weight, cocoon shell percentage, raw silk %, denier and neatness followed by parents vs. hybrids for four characters viz., hatching %, yield/10,000 larvae by weight, cocoon weight and filament length.

Two multivoltine breeds BL_{67} and BL_{68} and one bivoltine breed, CSR_2 were found to be good having general combining ability. Usually, *B. mori* breeds possessing high GCA effects are known to manifest high hybrid vigour because of additive effects and additive × additive type of gene interaction (Ravindra Singh *et al.*, 2003). The performance of hybrids depends upon genetic divergence between the parents and their proper selection. Selection of parents depends not only on genotype itself but also on its performance evaluated over a series of environmental conditions (Hallauver, 1987). In *B. mori*, most of the economic characters are influenced by environmental factors like temperature, humidity, nutrition and rearing techniques (Kogure, 1933; Arai and Ito, 1967; Horie *et al.*, 1967).

In the present study, the significance of G× E interaction for different characters and mean square for environment suggests that two multivoltine breeds BL_{67} and BL_{68} and one bivoltine CSR_2 can be utilized as breeding resource materials in the development of superior silkworm breeds.

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(Received 10 November 2006; accepted 15 May 2007)

ENTOMON **32(2)**: 149–151 (2007)
Short Communication No. ent.32212

Effect of temperature on the development of forensically important blowfly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)

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ABSTRACT: Development time of immature stages of the blowfly *Chrysomya megacephala* (Fabricius) was studied in the laboratory at four constant temperatures (22 °C, 25 °C, 28 °C, 30 °C). The development periods from oviposition to adult emergence were inversely related to temperature and ranged from 6.3 days at 30 °C to 15.5 days at 22 °C. © 2007 Association for Advancement of Entomology

KEYWORDS: *Chrysomya megacephala*, forensic entomology, development time

The presence of some species of insects, including their immature stages, can provide information about the location, time and conditions of cadavers and hence forensic entomologists make use of the same in crime investigations. Blowflies are the most important forensic indicators because they are usually the first to colonize carcass, often within minutes or even seconds of exposure (Greenberg, 1991). Bharti and Singh (2003) observed that out of the five known forensically important blowfly species in Punjab, *Chrysomya megacephala* and *C. rufifacies* were associated with carcasses throughout the year. They concluded that these files can withstand extreme temperature fluctuations and thus can help to calculate the Post Mortem Interval (PMI) in all the seasons of the year. The development of *C. megacephala* at different temperatures (22 °C, 25 °C, 28 °C and 30 °C), was hence studied in the laboratory.

Adults of *C. megacephala* were collected from animal carcasses found in the field at Patiala (Punjab, India). Adults were allowed to feed, mate and oviposit in rearing chambers (2′x2′x2′). A piece of goat liver placed on moistened filter paper in a Petri dish provided in the rearing chambers served as the oviposition medium. Eggs laid were transferred into a 200 ml glass jar the bottom of which was filled up to 5 cm height with moistened saw dust to prevent desiccation. Mutton was provided as food for the emerging larvae. The jars were closed with muslin cloth and kept in incubators maintained at varying temperatures. The jars were frequently checked to record the

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TABLE 1. Development period of *Chrysomya megacephala* at various temperatures

	Duration in hours						
Temp	Egg	1st Instar	2nd Instar	3rd Instar	Post-feeding	Pupa	Egg to Adult
22 °C	19. 0	18.0	33.0	44.0	92.0	168.0	374 (15.5 days)
25 °C	17.0	16.0	26.0	40.0	81.0	119.0	299 (12.4 days)
28 °C	15.0	14.0	21.0	25.0	34.0	97.0	206 (8.5 days)
30 °C	12.2	12.0	16.0	18.1	23.0	72.0	153.4 (6.3 days)

emergence of different life stages and thus to work out duration of different immature stages of the insect.

To calculate the PMI of a body with the help of immature stages we must have knowledge about the development pattern of the fly in question at different temperature regimes. Very few workers have systematically studied the development pattern of *C. megacephala* under different temperature regimes. Wijesundra (1957) reported that the eggs hatch at 27 °C in 9–10 hrs. Nishida (1984) found that the duration of post feeding stage lasted for 23 hrs at 30 °C. Wells and Kurahashi (1994) found that the development period at 27 °C was 9.75 days. The age grading of the immature stages of this species at different temperatures has been done for the first time and the data are presented in Table 1. Age grading of insects will have direct bearing on the post mortem interval of a body, and can even help the forensic entomologist to estimate the temperature of the place where the body was kept (Higley and Haskell, 2002).

Thus the data generated from this study will be useful for making forensically relevant conclusions, when immature stages of *C. megacephala* are found associated with a dead body.

ACKNOWLEDGEMENTS

Financial assistance rendered by CSIR, New Delhi, vide grant no. 9/140(137)/2004-EMRI is acknowledged.

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(Received 9 November 2006; accepted 15 May 2007)

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